2-Arylmethyl-1,4-benzoquinones. II. Novel Inhibitors of Platelet Aggregation: Synthesis and Pharmacological Evaluation[1)]

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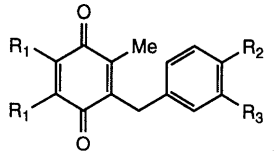
Two new series of 2-arylmethyl-1,4-benzoquinones (2 and 3) were synthesized for evaluation of their pharmacological activities. These compounds showed significant inhibition of platelet aggregation and some of them possessed a protective effect against endothelial cell injury. Structure–activity relationship studies indicated that 2b, 2d and 3b are potent inhibitors of platelet aggregation induced by arachidonic acid (AA) with an IC_{50} in the range of 1—10 μg/ml. Among them, 3b showed a significant inhibitory activity against endothelial cell injury caused by hydrogen peroxide (H_2O_2) at 1 μM.

Key words 1,4-benzoquinone; anti-platelet aggregation; arachidonic acid; hydrogen peroxide; endothelial cell

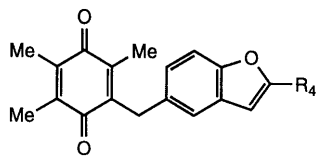
The prevalence of occlusive vascular and platelet-related diseases continues to stimulate research into new therapies for these conditions. We have recently reported the synthesis and pharmacological evaluation of a series of 2-phenylmethyl-1,4-benzoquinone derivatives.[1)] These compounds were found to be inhibitors of platelet aggregation induced by arachidonic acid (AA) and a structure–activity relationships (SAR) study led to the identification of 3-[3-(3,5,6-trimethyl-1,4-benzoquinon-2-ylmethyl)phenyl]propionic acid (**1**) as the most potent inhibitor among them. It is well known that thromboxane A_2 (TXA_2), a major arachidonic acid metabolite in platelets, is a potent inducer of platelet aggregation and constrictor of vascular and respiratory smooth muscles. It has also been implicated as an important mediator in a variety of diseases such as myocardial infarction, stroke and anaphylaxis.[2)] During our study, (±)-7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenylheptanoic acid (seratrodast, AA-2414), which possesses similar functional groups in the molecule, was reported as a novel type of eicosanoid receptor antagonist, and it was suggested that both a benzoquinonyl moiety and a carboxylic acid moiety, with appropriate separation, were essential for exhibiting significant activity.[3)] We therefore became interested in more fully investigating the structural requirements for anti-platelet aggregation activity associated with this class of compounds. As part of an effort to examine the effect of modifications on the phenyl ring of **1**, two new series of benzoquinone derivatives (**2** and **3**), which contain a phenoxyacetic acid or benzofuran-2-carboxylic acid moiety in the molecule, were targeted for synthesis and biological evaluation. (Fig. 1) In this paper, we report the synthesis and pharmacological evaluation of new 2-arylmethyl-1,4-benzoquinone derivatives (**2** and **3**) which exhibit marked anti-platelet aggregation activity.

Chemistry

Synthetic procedures for the target compounds are summarized in Charts 1—3. The 3,5,6-trimethyl-2-phenylmethyl-1,4-benzoquinones (**2a**—**c**) were prepared from the



1: R_2=H, R_3=$(CH_2)_2COOH$
2: R_2,R_3=H, OCH_2COR_4



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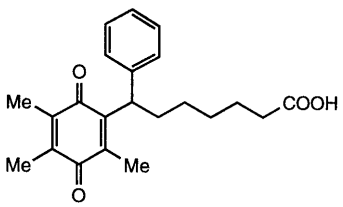
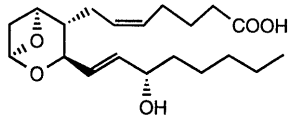


seratrodast (AA-2414)



TXA_2

Fig. 1

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benzaldehyde (**4**). Reaction of **4** with Grignard reagent prepared from 4-benzyloxybromobenzene afforded **5**, which was transformed to the phenol (**6**) by reduction with triethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst and subsequent hydrogenolysis. Acetylation of **6** and oxidation with cerium ammonium nitrate (CAN)[4] afforded the benzoquinone (**7**). Hydrolysis of **7**, followed by alkylation with *tert*-butyl bromoacetate and acidic hydrolysis, gave the carboxylic acid (**2a**) (Y=OH). Compound **2a** was transformed to the ester (**2b**) or amide (**2c**) by condensation with the appropriate alcohol or amine (see Chart 1).

The 5,6-dimethoxy-3-methyl-2-phenylmethyl-1,4-benzoquinones (**2g, h**), in which the substitution on the quinonyl moiety of (**2a, b**) was varied, were prepared from the salicylaldehyde (**8**). Coupling of **8** with the Grignard reagent, followed by acetylation, gave the diacetate (**9**), which was converted to the phenol (**10**) by a similar procedure to that used for the synthesis of **6**. Alkylation of **10** with *tert*-butyl bromoacetate afforded **11** as the major product. In this case, the less hindered phenolic hydroxy group was selectively alkylated by using one equivalent of the reagent. The obtained phenol derivative (**11**) was transformed to the benzoquinone derivative (**2g**) (Y=OH) by oxidation with Fremy's salt or by catalytic air oxidation[5] and subsequent acidic hydrolysis. Compound **2g** was converted to the ester (**2h**) by condensation with alcohol.

The 3,5,6-trimethyl-2-(benzofuran-5-yl)methyl-1,4-benzoquinones (**3a—d**) were synthesized by the method illustrated in Chart 3. Reaction of **6** with hexamethylenetetramine in trifluoroacetic acid gave the aldehyde (**12**).[6] Condensation of **12** with diethyl bromomalonate afforded the benzofuran (**13a**) (Y=OEt),[7] which was converted to

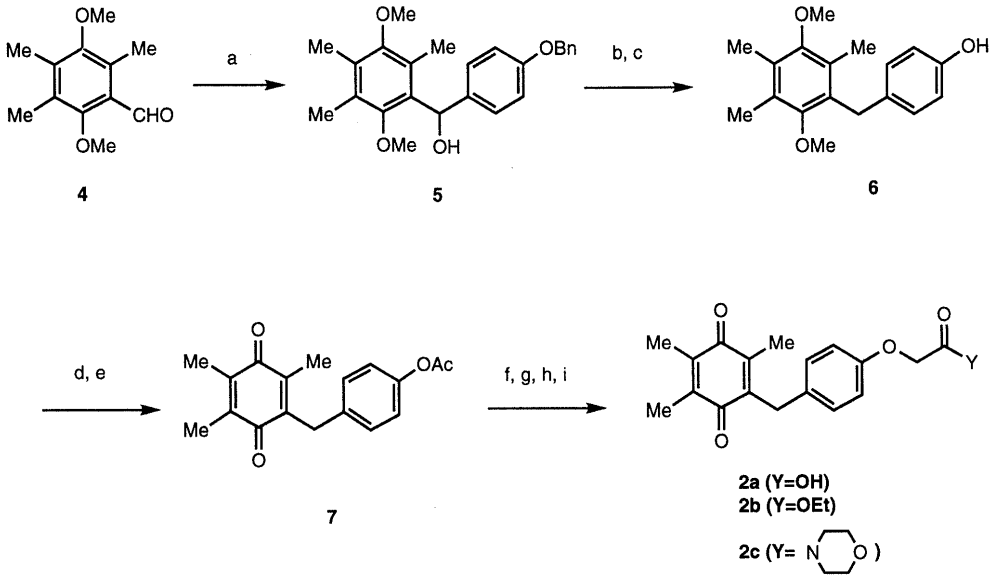


Chart 1

a: 4-benzyloxybromobenzene, Mg b: Et_3SiH, TMSOTf c: H_2, Pd–C d: Ac_2O, pyridine, DMAP e: CAN f: $NaHCO_3$, H_2O, MeOH g; $BrCH_2CO_2$*tert*-Bu, K_2CO_3 h: HCOOH i: HOR or HNR_2, DCC

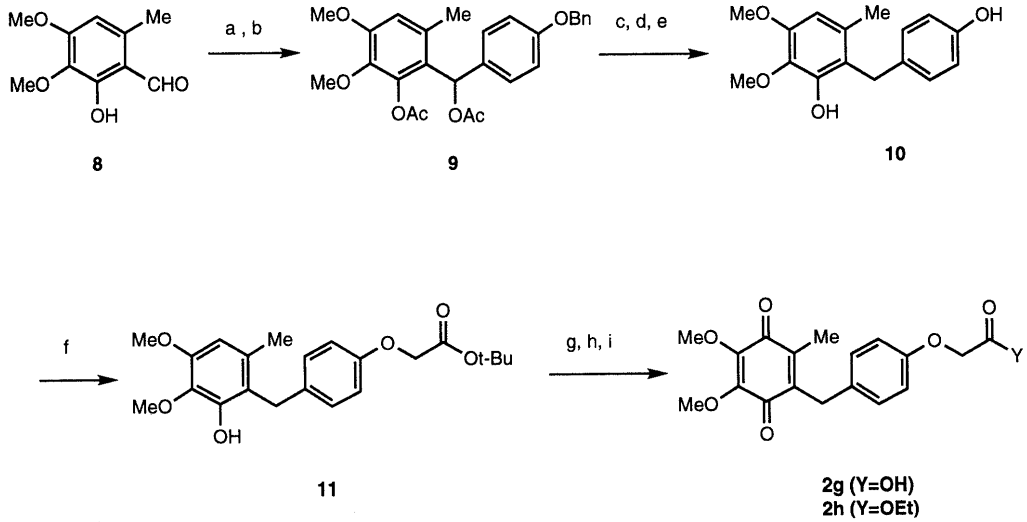


Chart 2

a: 4-benzyloxybromobenzene, Mg b: Ac_2O, pyridine, DMAP c: Et_3SiH, TMSOTf d: Pd–black, H_2 e: 1 N NaOH f: $BrCH_2CO_2$*tert*-Bu, K_2CO_3 g: salcomine, O_2 h: HCO_2H i: ROH, DMAP, DCC

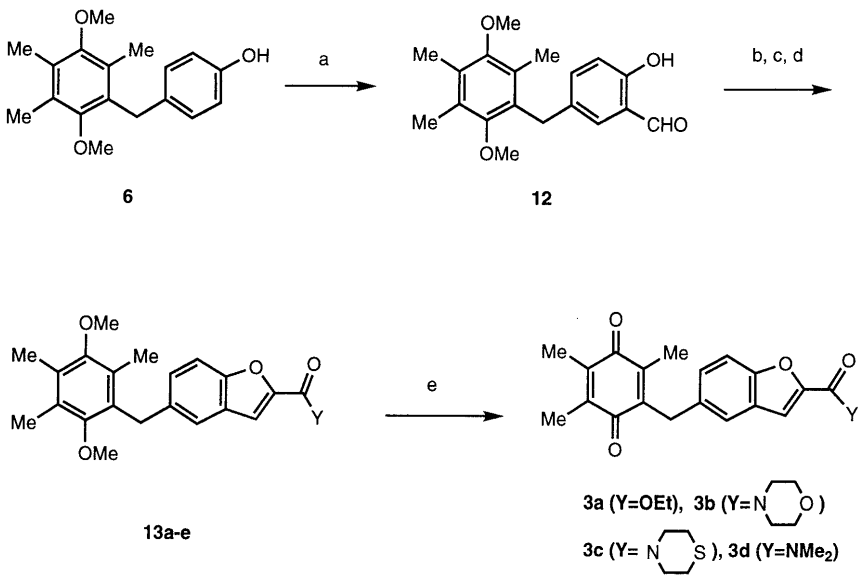
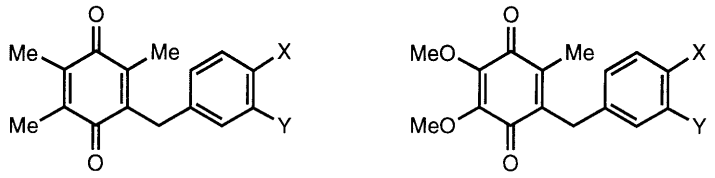


Chart 3

a: hexamethylenetetramine, CF_3CO_2H b: $BrCH(CO_2Et)_2$, K_2CO_3 c: 1 N NaOH d: HNR_2, DCC e: CAN

Table 1. Physical Data for Target Compounds (**2a—j**)



2a—f **2g—j**

Compound	X	Y	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
2a	OCH_2COOH	H	74	168—170	$C_{18}H_{18}O_5$	68.78	5.77		68.68	5.72	
2b	OCH_2COOEt	H	83	74—75	$C_{20}H_{22}O_5$	70.16	6.48		70.05	6.30	
2c	OCH_2CON O	H	64	114—116	$C_{22}H_{25}NO_5$	384.1573[c]			384.1596[c]		
2d	H	OCH_2COOH	73	129—131	$C_{18}H_{18}O_5$	68.78	5.77		68.70	5.75	
2e	H	OCH_2COOEt	70	[b]	$C_{20}H_{22}O_5$	70.16	6.48		69.92	6.30	
2f	H	OCH_2CON O	57	100—101	$C_{22}H_{25}NO_5$	384.1573[c]			384.1570[c]		
2g	OCH_2COOH	H	72	140—142	$C_{18}H_{18}O_7$	346.1053[c]			346.1036[c]		
2h	OCH_2COOEt	H	65	[b]	$C_{20}H_{22}O_7$	374.1366[c]			374.1347[c]		
2i	H	OCH_2COOH	81	[a]	$C_{18}H_{18}O_7$	346.1053[c]			346.1042[c]		
2j	H	OCH_2COOEt	55	[b]	$C_{20}H_{22}O_7$	374.1366[c]			374.1367[c]		

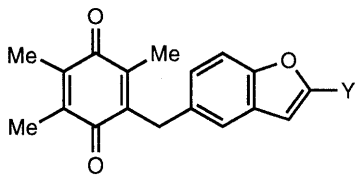
a) Not measured because the compound is extremely hygroscopic. *b*) Obtained as an oil. *c*) Determined by high-resolution mass spectrometry.

the benzoquinone (**3a**) by oxidation with CAN. Compound **13a** could be transformed to the various amides (**13b—d**) by hydrolysis and subsequent condensation with appropriate amines in the usual way. These amides could be converted to the corresponding benzoquinones (**3b—d**) by the same method as that used for the synthesis of **3a**.

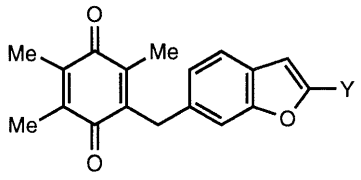
Regioisomers of the above-mentioned compounds were also synthesized similarly. Chemical structures of the synthesized compounds were determined on the basis of spectroscopic data [infrared (IR), proton magnetic resonance (^{1}H-NMR) and mass spectra (MS)] and elemental analyses. The physical data are summarized in Tables 1 and 2.

Pharmacological Evaluation Anti-platelet aggregation activities of the benzoquinone derivatives described above were measured in terms of the ability to inhibit platelet aggregation induced by collagen, AA and adenosine diphosphate (ADP), as described by Born.[8] The results are displayed in Table 3 as IC_{50} values (the concentration needed to inhibit platelet aggregation by 50%). These compounds are analogues of the 2-phenylmethyl-1,4-benzoquinone **1**, in which modifications were made on the phenyl ring. The phenoxyacetic acid (**2a**) ($R_2=OCH_2CO_2H$, $R_3=H$) was found to show 10-fold less potent

Table 2. Physical Data for Target Compounds (**3a**—**f**)



3a—**d**



3e, **f**

Compound	Y	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd			Found		
					C	H	N	C	H	N
3a	COOEt	76	126—127	$C_{21}H_{20}O_5$	71.58	5.72		71.51	5.76	
3b	CON⟨⟩O	66	128—129	$C_{23}H_{23}NO_5$	70.21	5.89	3.56	70.14	5.98	3.50
3c	CON⟨⟩S	72	164—166	$C_{23}H_{23}NO_4S$	67.46	5.66	3.42	67.16	5.53	3.29
3d	$CONMe_2$	60	123—126	$C_{21}H_{21}NO_4$	71.78	6.02	3.99	71.57	5.89	3.79
3e	COOEt	41	157—159	$C_{21}H_{20}O_5$	71.58	5.72		71.03	5.61	
3f	CON⟨⟩O	73	130—132	$C_{23}H_{23}NO_5$	70.21	5.89	3.56	70.18	5.96	3.52

Table 3. Pharmacological Evaluation of **2a**—**e** and **3a**—**f**

Compound	Anti-platelet aggregation (IC_{50}: μg/ml)			LDH release (inhibition %)	
	Collagen	AA	ADP	10 μM	1 μM
2a	>200	20.4	>200	48	13
2b	3.8	4.2	>200	—	9
2c	>200	74	>200	2	—
2d	95	8.8	>200	37	—
2e	210	42	>200	—	29
2g	>200	60	>200	—	—
3a	>200	33	>200	—	18
3b	>200	1.6	>200	—	65
3c	>200	>200	>200	—	12
3d	>200	>200	>200	—	15
3e	>200	96	>200	—	20
3f	>200	72	>200	—	16
1	>200	3.4	>200	78	35

activity against platelet aggregation induced by AA ($IC_{50}=20\,\mu$g/ml) than the parent compound **1**.

This compound was inactive against collagen- or ADP-induced aggregation ($IC_{50}>200\,\mu$g/ml). A regio-isomer **2d** ($R_2=H$, $R_3=OCH_2CO_2H$) was found to be more potent than **2a** ($IC_{50}=8.8\,\mu$g/ml). These results prompted us to investigate the structure–activity relationships for this class of compounds. Modifications of the carboxylic acid moiety of **2d**, such as conversion to the ester (**2e**) ($R_2=H$, $R_3=OCH_2CO_2Et$) ($IC_{50}=42\,\mu$g/ml), resulted in a reduction of activity. In contrast, similar conversion of the regio-isomer (**2a**) to the corresponding ester **2b** ($R_2=OCH_2CO_2Et$, $R_3=H$) ($IC_{50}=4.2\,\mu$g/ml) improved the activity. In addition, conversion of **2a** to the morpholine amide **2c** ($IC_{50}=74\,\mu$g/ml) resulted in a reduction of activity. Modification of the benzoquinone ring, such as replacement of the methyl group of **2a** ($R_1=Me$) by a methoxy group (**2g**) ($R_1=MeO$), resulted in loss of activity ($IC_{50}=60\,\mu$g/ml). We next investigated the effect of ring annelation of the phenoxyacetic acids to the corresponding benzofuran-2-carboxylic acids. Similar modifications have been reported for thromboxane synthetase inhibitors[7] and the constraint imposed by the rigid benzofuran ring fixes the carboxyl group in an optimal spatial position. The 5-substituted benzofuran-2-carboxylate **3a** ($R_4=CO_2Et$) ($IC_{50}=33\,\mu$g/ml) was 10-fold less potent than the parent compound **1** against AA-induced platelet aggregation.

The 6-substituted analogue **3e** ($R_4=CO_2Et$) ($IC_{50}=96\,\mu$g/ml) was also less effective. We could not synthesize the carboxylic acid derivatives ($R_4=CO_2H$) by the usual acidic hydrolysis of the esters (**3a**) or (**3e**). In this series, modifications of the carboxylic acid moiety, such as conversion to the morpholine amides (**3b**) ($IC_{50}=1.6\,\mu$g/ml) and (**3f**) ($IC_{50}=72\,\mu$g/ml), improved the activity. In contrast, the dimethylamide (**3d**) ($IC_{50}=>200\,\mu$g/ml) and the thiomorpholine amide (**3c**) ($IC_{50}=>200\,\mu$g/ml) were found to lack the activity. These results suggest that the oxygen atom in the morpholine ring may be important for activity.

In summary, the results of chemical modification suggest that (1) the C(3)-carbon atom in 3-phenylpropionic acid moiety of **1** can be replaced by oxygen with retention of the activity and (2) a substantial potency enhancement is caused by ring annelation to the corresponding benzofuran analogues, as in the case of the morpholine amide **3b**.

An assay for protective activity against endothelial cell injury caused by hydrogen peroxide was also conducted since the generation of free radicals is observed in some cardiovascular diseases and it is believed that such radical species may damage the cardiac tissues and the vessels. Selected compounds in this series were evaluated in this screening assay and their protective activities were evaluated as inhibition (%) of lactate dehydrogenase (LDH) release from endothelial cells as described by Abe

et al.[9] Compound **3b** was found to show significant protective activity in this screening test (65% inhibition at 1 μM). Thus, new candidates for our project have been found. Further SAR studies and pharmacological experiments are under way and the results will be published elsewhere.

Experimental

Melting points were determined on a Yanaco melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a JEOL JNM-GX270 spectrometer, using tetramethylsilane as an internal standard, and IR spectra were obtained with either a Hitachi 260-10 or a Nicolet 5DX instrument. Elemental analyses were performed on a Perkin–Elmer 240B elemental analyzer. MS were obtained with a Hitachi M80 or a JMS-AX500 instrument with a direct inlet system.

1-(4-Benzyloxyphenyl)-1-(2,5-dimethoxy-3,4,6-trimethylphenyl)methanol (5) A solution of 2,5-dimethoxy-3,4,6-trimethylbenzaldehyde (**4**) (7.05 g, 33.90 mmol) in tetrahydrofuran (THF) (100 ml) was added to a solution of Grignard reagent [prepared from 4-(benzyloxy)bromobenzene (5.30 g, 58.17 mmol) and magnesium (1.70 g, 69.96 mmol)] in THF (200 ml) under ice-cooling, followed by stirring at room temperature for 2 h. The reaction mixture was poured into water and extracted with ether. The organic solution was washed with water, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (5 : 1) to afford **5** (10.42 g, 26.58 mmol, 78.4%) as a colorless powder, mp 86—87.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.13 (3H, s), 2.21 (3H, s), 2.26 (3H, s), 3.14 (3H, s), 3.67 (3H, s), 4.29 (1H, d), 5.04 (2H, s), 6.00 (1H, d), 6.92 (2H, d), 7.10—7.50 (7H, m). IR (KBr): 3450, 1509, 1241 cm^{-1}. MS *m/z*: 392 (M^+). *Anal.* Calcd for $C_{25}H_{28}O_4$: C, 76.50; H, 7.19. Found: C, 76.43; H, 7.03.

By a similar procedure, 1-(3-benzyloxyphenyl)-1-(2,5-dimethoxy-3,4,6-trimethylphenyl)methanol was synthesized from 3-(benzyloxy)bromobenzene and 2,5-dimethoxy-3,4,6-trimethylbenzaldehyde. Colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 2.11 (3H, s), 2.22 (3H, s), 2.27 (3H, s), 3.05 (3H, s), 3.67 (3H, s), 4.28 (1H, d), 5.04 (2H, s), 5.99 (1H, d), 6.75—7.05 (3H, m), 7.15—7.45 (6H, m). IR ($CHCl_3$): 3400, 1594, 1450 cm^{-1}. HRMS: Calcd for $C_{25}H_{28}O_4$: 392.1988. Found: 392.1981.

4-(2,5-Dimethoxy-3,4,6-trimethylbenzyl)phenol (6) A solution of **5** (6.00 g, 15.30 mmol) in methylene chloride (100 ml) was added to a solution of triethylsilane (2.14 g, 18.45 mmol) and TMSOTf (170 mg, 0.77 mmol) in methylene chloride (200 ml) under ice-cooling over 30 min, followed by stirring at the same temperature for 1 h. The reaction mixture was washed with water, dried and concentrated to afford 2-(4-benzyloxybenzyl)-1,4-dimethoxy-3,5,6-trimethylbenzene (5.70 g, 15.16 mmol, 99%). A solution of this compound (5.70 g) in ethanol (50 ml) was added to a suspension of 5% palladium–C in ethanol (250 ml), followed by stirring under a hydrogen gas stream at room temperature for 16 h. After filtration, the filtrate was concentrated under reduced pressure and the residue was purified by column chromatography with hexane–ethyl acetate (4 : 1) to afford **6** (4.05 g, 14.16 mmol, 93.4%) as a colorless powder, mp 134—135 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.10 (3H, s), 2.21 (6H, s), 3.54 (3H, s), 3.64 (3H, s), 3.98 (2H, s), 4.69 (1H, s), 6.68 (2H, d), 6.93 (2H, d). IR (KBr): 3300, 1516, 1456 cm^{-1}. MS *m/z*: 286 (M^+). *Anal.* Calcd for $C_{18}H_{22}O_3$: C, 75.50; H, 7.74. Found: C, 75.39; H, 7.54.

By a similar procedure, 3-(2,5-dimethoxy-3,4,6-trimethylbenzyl)phenol was synthesized. Colorless powder, mp 82—84 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.10 (3H, s), 2.21 (6H, s), 3.55 (3H, s), 3.64 (3H, s), 4.01 (2H, s), 6.45—6.75 (3H, m), 7.10 (1H, s). MS *m/z*: 286 (M^+). *Anal.* Calcd for $C_{18}H_{22}O_3$: C, 75.50; H, 7.74. Found: C, 75.42; H, 7.51.

4-(3,5,6-Trimethyl-1,4-benzoquinon-2-ylmethyl)phenyl Acetate (7) A solution of **6** (500 mg, 1.75 mmol), acetic anhydride (428 mg), pyridine (828 mg) and 4-dimethylaminopyridine (DMAP) (43 mg) in methylene chloride (30 ml) was stirred at room temperature for 14 h. The reaction mixture was washed with 5% hydrochloric acid and brine, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (6 : 1) to afford 4-(2,5-dimethoxy-3,4,6-trimethylbenzyl)phenyl acetate (570 mg, 1.74 mmol, 99%). This compound (527 mg, 1.61 mmol) was dissolved in a mixture of acetonitrile (75 ml) and water (25 ml), then CAN (2.20 g, 4.01 mmol) was added at room temperature, followed by stirring at the same temperature for 30 min. The reaction mixture was diluted with water and extracted with ether. The organic solution was washed with water, dried and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography with hexane–ethyl acetate (4 : 1) to afford **7** (403 mg, 1.35 mmol, 84.2%) as a yellow powder, mp 88—89 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.02 (6H, s), 2.11 (3H, s), 2.27 (3H, s), 3.85 (2H, s), 6.97 (2H, d), 7.18 (2H, d). IR (KBr): 1748, 1646 cm^{-1}. FAB-MS *m/z*: 299 ($M^+ + 1$). *Anal.* Calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.38; H, 6.04.

By a similar procedure, 3-(3,5,6-trimethyl-1,4-benzoquinon-2-ylmethyl)phenyl acetate was synthesized. Yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 2.01 (6H, s), 2.09 (3H, s), 2.27 (2H, s), 3.86 (2H, s), 6.80—7.15 (3H, m), 7.26 (1H, t). IR (KBr): 1765, 1643 cm^{-1}. HRMS: Calcd for $C_{25}H_{28}O_4$: 298.1205. Found: 298.1221.

4-(3,5,6-Trimethyl-1,4-benzoquinon-2-ylmethyl)phenoxyacetic Acid (2a) A solution of **7** (380 mg, 1.28 mmol) in a mixture of saturated aqueous $NaHCO_3$ solution (20 ml), water (20 ml) and methanol (80 ml) was stirred at room temperature for 1 h. The reaction mixture was diluted with water, acidified and extracted with ether. The extract was washed with water, dried and concentrated to obtain 4-(3,5,6-trimethyl-1,4-benzoquinon-2-ylmethyl)phenol (278 mg, 1.09 mmol, 85.2%). The product (150 mg, 0.59 mmol) was dissolved in acetone (20 ml), then anhydrous K_2CO_3 (485 mg) and *tert*-butyl bromoacetate (229 mg, 1.17 mmol) were added, followed by stirring at room temperature for 14 h. After filtration, the filtrate was concentrated under reduced pressure and the residue was purified by silica-gel column chromatography with hexane–ethyl acetate (6 : 1) to afford *tert*-butyl 4-(3,5,6-trimethyl-1,4-benzoquinon-2-ylmethyl)phenoxyacetate (145 mg, 0.39 mmol, 66.1%). The product (130 mg, 0.35 mmol) was dissolved in formic acid (10 ml), followed by stirring at room temperature for 1 h. The reaction mixture was concentrated to afford **2a** (82 mg, 0.26 mmol, 74.3%) [recrystallized from hexane–ether (1 : 3)]. ^{1}H-NMR ($CDCl_3$) δ: 2.01 (6H, s), 2.09 (3H, s), 3.80 (2H, s), 4.62 (2H, s), 6.82 (2H, d), 7.12 (2H, d). IR (KBr): 3430, 1734, 1644 cm^{-1}. FAB-MS *m/z*: 315 ($M^+ + 1$).

By a similar procedure, 3-(3,5,6-trimethyl-1,4-benzoquinon-2-ylmethyl)phenoxyacetic acid was synthesized. ^{1}H-NMR ($CDCl_3$) δ: 2.02 (6H, s), 2.08 (3H, s), 3.84 (2H, s), 4.65 (2H, s), 6.65—6.95 (3H, m), 7.20 (1H, t). IR (KBr): 3500, 1736, 1642 cm^{-1}. FAB-MS *m/z*: 315 ($M^+ + 1$).

4-[4-(3,5,6-Trimethyl-1,4-benzoquinon-2-ylmethyl)phenoxyacetyl]morpholine (2c) A solution of **2a** (35 mg, 0.11 mmol) and morpholine (13 mg, 0.15 mmol) in methylene chloride (20 ml) was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (38 mg, 0.20 mmol), followed by stirring at room temperature for 2 h. The reaction mixture was washed with water, dried and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (1 : 3) to afford **2c** (26 mg, 0.07 mmol, 63.6%).

Compounds **2b**, **2e** and **2f** were synthesized by a similar procedure to that described above. The physical data are listed in Table 1.

1-(2-Acetoxy-3,4-dimethoxy-6-methylphenyl)-1-(4-benzyloxyphenyl)-methyl Acetate (9) A solution of **8** (1.11 g, 5.66 mmol) in THF (40 ml) was added to a solution of Grignard reagent [prepared from 4-(benzyloxy)bromobenzene (4.48 g) and magnesium (440 mg)] in THF under ice-cooling, followed by stirring at room temperature for 5 h. The reaction mixture was worked up in the usual way and the crude product obtained was purified by silica-gel column chromatography with hexane–ethyl acetate (3 : 1) to afford 1-(4-benzyloxyphenyl)-1-(2-hydroxy-3,4-dimethoxy-6-methylphenyl)methanol (780 mg, 2.05 mmol, 36.2%). This compound (780 mg, 2.05 mmol) was dissolved in a mixture of acetic anhydride (837 mg), pyridine (730 mg) and DMAP (20 mg) in methylene chloride (100 ml).

The mixture was stirred at room temperature for 12 h, then washed with 5% hydrochloric acid and water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (3 : 1) to afford **9** (685 mg, 1.48 mmol, 72.2%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 2.11 (3H, s), 2.20 (3H, s), 2.33 (3H, s), 3.80 (3H, s), 3.86 (3H, s), 5.04 (2H, s), 6.63 (1H, s), 6.90 (2H, d), 7.00—7.20 (3H, m), 7.20—7.50 (5H, m). IR ($CHCl_3$): 1749, 1732 cm^{-1}. HRMS: Calcd for $C_{27}H_{28}O_7$: 464.1835. Found: 464.1855.

By a similar procedure, 1-(2-acetoxy-3,4-dimethoxy-6-methylphenyl)-1-(3-benzyloxyphenyl)methyl acetate was synthesized from **8** and 3-(benzyloxy)bromobenzene. Colorless powder, mp 99—100 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.11 (3H, s), 2.19 (3H, s), 2.31 (3H, s), 3.79 (3H, s), 3.86 (3H, s), 5.02 (2H, s), 6.62 (1H, s), 6.70—6.90 (3H, m), 7.10—7.45 (7H, m). IR (KBr): 1744 cm^{-1}. MS *m/z*: 464 (M^+). HRMS: Calcd for

$C_{27}H_{28}O_7$: 464.1835. Found: 464.1847.

4-(2-Hydroxy-3,4-dimethoxy-6-methylbenzyl)phenol (10) A solution of **9** (680 mg, 1.47 mmol) in methylene chloride (40 ml) was added to a solution of triethylsilane (255 mg, 2.20 mmol) and TMSOTf (16 mg) in methylene chloride (100 ml) at room temperature, followed by stirring at the same temperature for 40 min. The reaction mixture was poured into water and extracted with ether. The extract was washed with water, dried and concentrated. The crude product was dissolved in a suspension of Pd–black (100 mg) in dioxane (50 ml) and stirred under a hydrogen gas stream at room temperature for 12 h. After filtration, the filtrate was concentrated under reduced pressure and the residue was purified by silica-gel column chromatography with hexane–ethyl acetate (3 : 1) to afford 4-(2-acetoxy-3,4-dimethoxy-6-methylbenzyl)phenol (412 mg, 1.30 mmol, 88.4%).

This compound was dissolved in a mixture of 1 N aqueous sodium hydroxide (10 ml) and THF (10 ml), followed by stirring at room temperature for 12 h. The reaction mixture was acidified with concentrated hydrochloric acid and extracted with ether. The extract was washed with water, dried and concentrated to afford **10** (253 mg, 0.92 mmol, 70.8%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 2.18 (3H, s), 3.84 (3H, s), 3.89 (3H, s), 3.91 (2H, s), 4.71 (1H, s), 5.88 (1H, s), 6.31 (1H, s), 6.69 (2H, d), 7.02 (2H, d). IR (KBr): 3332, 1616 cm^{-1}. HRMS: Calcd for $C_{16}H_{18}O_4$: 274.1205. Found: 274.1223.

By a similar procedure, 3-(2-hydroxy-3,4-dimethoxy-6-methylbenzyl)-phenol was synthesized. Colorless powder, mp 86—87.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.18 (3H, s), 3.85 (3H, s), 3.90 (3H, s), 3.95 (2H, s), 4.59 (1H, s), 5.88 (1H, s), 6.32 (1H, s), 6.50—6.85 (3H, m), 7.11 (1H, t). IR ($CHCl_3$): 3520, 3330, 1590 cm^{-1}. HRMS: Calcd for $C_{16}H_{18}O_4$: 274.1205. Found: 274.1232.

***tert*-Butyl 4-(2-Hydroxy-3,4-dimethoxy-6-methylbenzyl)phenoxy-acetate (11)** A solution of **10** (80 mg, 0.29 mmol) and *tert*-butyl bromoacetate (60 mg, 0.31 mmol) in acetone (5 ml) was added to a suspension of anhydrous potassium carbonate (121 mg) in acetone (15 ml), followed by stirring at room temperature for 12 h. After filtration, the filtrate was concentrated and the residue was purified by silica-gel column chromatography with hexane–ethyl acetate (3 : 1) to afford **11** (38 mg, 0.10 mmol, 34.5%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 2.18 (3H, s), 3.84 (3H, s), 3.89 (3H, s), 3.92 (2H, s), 4.45 (2H, s), 5.87 (1H, s), 6.30 (1H, s), 6.77 (2H, d), 7.06 (2H, d). IR ($CHCl_3$): 3522, 1749, 1509 cm^{-1}. HRMS: Calcd for $C_{22}H_{28}O_6$: 388.1886. Found: 388.1872.

By a similar procedure, *tert*-butyl 3-(2-hydroxy-3,4-dimethoxy-6-methylbenzyl)phenoxyacetate was synthesized. Colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.17 (3H, s), 3.84 (3H, s), 3.89 (3H, s), 3.95 (2H, s), 4.45 (2H, s), 5.87 (1H, s), 6.30 (1H, s), 6.60—6.85 (3H, m), 7.14 (1H, t). IR ($CHCl_3$): 3522, 1749, 1590 cm^{-1}. HRMS: Calcd for $C_{22}H_{28}O_6$: 388.1886. Found: 388.1877.

4-(5,6-Dimethoxy-3-methyl-1,4-benzoquinon-2-ylmethyl)phenoxyacetic Acid (2g) A solution of **11** (30 mg, 0.08 mmol) in DMF (3 ml) was added to a suspension of salcomine (10 mg) in DMF (15 ml), followed by stirring under an oxygen gas stream at room temperature for 12 h. The reaction mixture was poured into water and extracted with ether. The extract was washed with water, dried and concentrated. The crude product obtained was purified by silica-gel column chromatography with hexane–ethyl acetate (3 : 1) to afford *tert*-butyl 4-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-ylmethyl)phenoxyacetate (21 mg, 0.05 mmol, 67.5%). This compound (45 mg, 0.11 mmol) was dissolved in formic acid (5 ml) and the reaction mixture was stirred at room temperature for 12 h. After concentration, the residue was washed with a mixture of hexane–ether (3 : 1) and filtered to afford **2g** (28 mg, 0.08 mmol, 72.3%). ^{1}H-NMR ($CDCl_3$) δ: 2.08 (3H, s), 3.79 (2H, s), 3.98 (3H, s), 3.99 (3H, s), 4.63 (2H, s), 6.83 (2H, s), 7.12 (2H, d). IR (KBr): 3566, 1736, 1709 cm^{-1}. MS *m/z*: 346 (M^+).

By a similar procedure, 3-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-ylmethyl)phenoxyacetic acid was synthesized. ^{1}H-NMR ($CDCl_3$) δ: 2.08 (3H, s), 3.81 (2H, s), 3.98 (3H, s), 3.99 (3H, s), 4.63 (2H, s), 6.65—6.90 (3H, s), 7.10—7.30 (1H, *t*-like). IR (KBr): 3425, 1733, 1645 cm^{-1}. MS *m/z*: 346 (M^+).

Compounds **2h** and **2j** were synthesized by a usual method and the physical data are listed in Table 1.

4-(2,5-Dimethoxy-3,4,6-trimethylbenzyl)salicylaldehyde (12) A solution of **6** (3.50 g, 12.24 mmol) and hexamethylenetetramine (2.23 g, 15.91 mmol) in trifluoroacetic acid (100 ml) was heated at 80 °C for 4 h and the reaction mixture was concentrated under reduced pressure. The residue was diluted with methylene chloride and the solution was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (5 : 1) to afford **12** (3.12 g, 9.94 mmol, 81.2%) as colorless powder, mp 91—92 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.11 (3H, s), 2.22 (6H, s), 3.58 (3H, s), 3.64 (3H, s), 4.02 (2H, s), 6.88 (1H, d), 7.20 (1H, s-like), 7.32 (1H, d-like), 9.78 (1H, s), 10.85 (1H, s). IR (KBr): 3800, 1659 cm^{-1}. HRMS: Calcd for $C_{19}H_{22}O_4$: 314.1518. Found: 314.1510.

By a similar procedure, 5-(2,5-dimethoxy-3,4,6-trimethylbenzyl)salicyl-aldehyde was synthesized. Colorless powder, mp 81—82 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.08 (3H, s), 2.21 (3H, s), 2.22 (3H, s), 3.56 (3H, s), 3.64 (3H, s), 4.07 (2H, s), 6.69 (1H, s), 6.78 (1H, d, J=7.9 Hz), 7.40 (1H, d, J=7.9 Hz), 9.81 (1H, s), 11.02 (1H, s). IR (KBr): 3850, 1668 cm^{-1}. HRMS: Calcd for $C_{19}H_{22}O_4$: 314.1518. Found: 314.1523.

Ethyl 5-(2,5-Dimethoxy-3,4,6-trimethylbenzyl)benzofuran-2-carboxyl-ate (13a) A solution of **12** (3.0 g, 9.55 mmol) and diethyl bromomalonate (4.57 g, 19.12 mmol) in methylethylketone (200 ml) was treated with potassium carbonate (5.27 g, 38.19 mmol), followed by stirring at room temperature for 1 h and refluxing for 40 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate and the solution was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (5 : 1) to afford **13a** (2.83 g, 7.41 mmol, 77.5%) as a colorless powder, mp 88—89 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, t), 2.12 (3H, s), 2.23 (6H, s), 3.55 (3H, s), 3.63 (3H, s), 4.15 (2H, s), 4.42 (2H, q), 7.20—7.50 (4H, m). IR (KBr): 1737 cm^{-1}. HRMS: Calcd for $C_{23}H_{26}O_5$: 382.1780. Found: 382.1772.

Ethyl 5-(3,5,6-Trimethyl-1,4-benzoquinon-2-ylmethyl)benzofuran-2-carboxylate (3a) A solution of **13a** (40 mg, 0.10 mmol) in a mixture of acetonitrile (3 ml) and water (1 ml) was treated with CAN (126 mg, 0.23 mmol), followed by stirring at room temperature for 30 min. The reaction mixture was diluted with water and extracted with ether. The extract was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (2 : 1) to afford **3a** (28 mg, 0.08 mmol, 75.9%). ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t), 2.02 (6H, s), 2.13 (3H, s), 3.96 (2H, s), 4.42 (2H, q), 7.20—7.60 (4H, m). IR (KBr): 1713, 1642 cm^{-1}. MS *m/z*: 352 (M^+).

By a similar procedure, ethyl 6-(3,5,6-trimethyl-1,4-benzoquinon-2-ylmethyl)benzofuran-2-carboxylate was synthesized. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, t), 2.02 (6H, s), 2.11 (3H, s), 4.00 (2H, s), 4.42 (2H, q), 7.16 (1H, d), 7.38 (1H, s), 7.47 (1H, s), 7.56 (1H, d). IR (KBr): 1714, 1639 cm^{-1}. MS *m/z*: 352 (M^+).

5-(2,5-Dimethoxy-3,4,6-trimethylbenzyl)benzofuran-2-carboxylic Acid (13e) A solution of **13a** (200 mg, 0.52 mmol) in a mixture of 0.5 N aqueous sodium hydroxide (3 ml) and THF (6 ml) was stirred under ice-cooling for 2 h and at room temperature for 12 h. The reaction mixture was diluted with water, acidified and extracted with ether. The extract was washed with water, dried and concentrated to afford **13e** (174 mg, 0.49 mmol, 93.9%) as a colorless powder, mp 174—176 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.13 (3H, s), 2.24 (6H, s), 3.58 (3H, s), 3.66 (3H, s), 4.17 (2H, s), 7.20—7.60 (4H, m). IR (KBr): 3800, 1704 cm^{-1}. MS *m/z*: 354 (M^+). *Anal.* Calcd for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26. Found: C, 71.30; H, 6.14.

By a similar procedure, 6-(2,5-dimethoxy-3,4,6-trimethylbenzyl)benzofuran-2-carboxylic acid was synthesized. Colorless powder, mp 175—177 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.11 (3H, s), 2.22 (3H, s), 2.23 (3H, s), 3.56 (3H, s), 3.65 (3H, s), 4.20 (2H, s), 7.05—7.65 (4H, m). IR (KBr): 3800, 1697 cm^{-1}. MS *m/z*: 354 (M^+). *Anal.* Calcd for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26. Found: C, 71.20; H, 6.19.

4-[5-(2,5-Dimethoxy-3,4,6-trimethylbenzyl)-2-benzofurancarbonyl]-morpholine (13b) A solution of **13e** (500 mg, 1.41 mmol) and morpholine (197 mg, 2.26 mmol) in methylene chloride (50 ml) was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (542 mg, 2.83 mmol), followed by stirring at room temperature for 4 h. The reaction mixture was washed with water, dried and concentrated. The crude product was purified by silica-gel column chromatography with hexane–ethyl acetate (1 : 2) to afford **13b** (420 mg, 0.99 mmol, 70.3%) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 2.12 (3H, s), 2.23 (6H, s), 3.56 (3H, s), 3.64 (3H, s), 3.65—3.95 (8H, s), 4.15 (2H, s), 7.15—7.45 (4H, m). IR ($CHCl_3$): 1612 cm^{-1}. MS *m/z*: 423 (M^+). *Anal.* Calcd for $C_{25}H_{29}NO_5$: C, 70.90; H, 6.90, N, 3.31. Found: C, 70.80; H, 6.79, N, 3.26.

4-[5-(3,5,6-Trimethyl-1,4-benzoquinon-2-ylmethyl)-2-benzofurancar-

bonyl]morpholine (3b) A solution of **13b** (400 mg, 0.95 mmol) in a mixture of acetonitrile (24 ml) and water (8 ml) was treated with CAN (1.70 g, 3.10 mmol), followed by stirring at room temperature for 3 h. The reaction mixture was diluted with water and extracted with ether. The extract was washed with water, dried and concentrated. The residue was purified by silica-gel column chromatography with hexane–ethyl acetate (1 : 1) to afford **3b** (245 mg, 0.62 mmol, 65.9%).

By a similar procedure, compounds **3c**—**f** were synthesized. The physical data are listed in Table 2.

Pharmacological Evaluation Platelet Aggregation Inhibitory Activity: Blood from male Japanese White rabbits was collected into plastic vessels containing 3.8% sodium citrate (1 volume with 9 volumes of blood). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation at $190 \times g$ for 7 min and then at $1500 \times g$ for 10 min, respectively. Platelet aggregation in PRP was measured by Born's standard turbidimetric procedure[8] using an eight-channel platelet aggregometer (PAM-8C, Mebanix, Tokyo, Japan). Activity of inhibitors (test compounds) was expressed as IC_{50} (μg/ml) values, *i.e.*, the doses required to inhibit the platelet aggregation response induced by collagen, arachidonic acid or ADP by 50%.

Protection Against Cell Injury Caused by Hydrogen Peroxide: A monolayer of endothelial cells (CPAE) in the stationary phase was washed with EBS (Earle's balanced salt solution) and incubated with EBS containing a test compound plus hydrogen peroxide (100 μM) for 6 h. After the incubation, LDH released into the medium was determined by a standard method.[9] Then the cells were stained with 0.02% erythrosine-B and the numbers of dead cells were counted from micrographs.

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