The ^1H NMR spectra of (+)-, (-)-, and (±)-**121d** in the presence of the chiral shift reagent Eu(facam)$_3$ were determined. The methine proton at the C_4 position of (±)-**121d** was observed at 4.87 ppm and 4.93 ppm in $CDCl_3$ containing (83 mg) of the shift reagent. This solution was spiked with pure (+)- and (-)-**121d**. The signal at 4.87 ppm increased in intensity when the (+) isomer was added and the signal at higher frequency responded to the addition of the (-) isomer, thus uniquely identifying each component. Additionally, (+)- and (-)-**121d** each exhibited only a single peak at the expected position. These studies confirmed optical purity of (+)- and (-)-**121d**.

The individual isomers (+)- and (-)-**121d** were converted to **54A** and **54B** by following methodology similar to compound **123a** and **123c**, respectively. (+)-**52** (60%): mp 174-176 °C; $[\alpha]^{23}_D$ (+) 121.6° (c = 0.55, $CHCl_3$). Anal. Calcd ($C_{23}H_{20}F_3N_3O_2S$) C, H, N. (-)-**52** (65%): mp 174-175 °C; $[\alpha]^{23}_D$ (-) 116.0° (c = 0.58, $CHCl_3$). Anal. Calcd ($C_{23}H_{20}F_3N_3O_2S$) C, H, N. **54A** (35%): white foam; $[\alpha]^{23}_D$ (+) 161° (c, 0.33, $CHCl_3$); HPLC (hexane/2-propanol 95:5) retention time 36.57 min. Anal. Calcd ($C_{23}H_{20}F_3N_3O_4S \cdot 0.8\ H_2O$) C, H, N. **54B** (37%): white foam; $[\alpha]^{23}_D$ (-) 147° (c = 0.33, $CHCl_3$); HPLC (hexane/2-isopropanol 95:5) retention time 41.16 min. Anal. Calcd ($C_{23}H_{20}F_3N_3O_4S \cdot 0.7\ H_2O$) C, H, N.

Diethyl 2-Cyano-1,4-dihydro-6-[(4-pyridylthio)methyl]-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate (123a). Pyridinium bromide perbromide (1 g, 31.2 mmol) was added to a solution of **121e** (1.14 g, 3.0 mmol) in $CHCl_3$ (15 mL) containing pyridine (0.24 mL) at -10 °C. The reaction mixture was stirred at that temperature for 1 h. Chloroform was distilled and the residue chromatographed with isopropyl ether to give 1.6 g of the bromide as a pale yellow foam. This was dissolved in THF (15 mL) and added to a solution of the anion [prepared from 4-mercaptopyridine (0.36 g) and NaH (60%, 1.37 g) in DMF (10 mL)]. The reaction mixture was stirred overnight at 23 °C. DMF was distilled, the residue was poured into water, and the dark gummy material was extracted with EtAC. EtOAc was evaporated and the residue chromatographed (EtAC/isopropyl ether 1:1) to yield 0.70 g (50%) of the desired material, mp 180-181 °C (red melt). Anal. ($C_{25}H_{22}F_3N_3O_4S$) C, H, N. Sulfone **123c** (foam): ^1H NMR δ 6.71 (s, 1 H, NH), 5.63 (s, 1 H, C4-H), 5.19 (d, J = 13 Hz, 1 H), 4.74 (d, J = 13.1 Hz, 1 H). Anal. ($C_{25}H_{22}F_3N_3O_6S$) C, H, N. Sulfoxide **123b** (foam): Anal. ($C_{25}H_{22}F_3N_3O_5S \cdot H_2O$) C, H, N.

Biological Methods. The procedures used to measure specific binding of [^3H]nitrendipine to calcium channels has been previously described by Taylor et al.[13] and Ehlert and co-workers.[23] Guinea pig left atrial contractile function and hemodynamic responses in the isolated Langendorff-perfused rat heart was evaluated by using methods described by Weishaar et al.[25] and Haleen and co-workers,[26] respectively. The ability of various compounds to block potassium-induced contractions in isolated rabbit aortic rings was examined by using the method of Kazda,[28] as modified by Weishaar et al.[29] The effect of various compounds on partially depolarized vascular muscle was studied by using the protocol previously described by Schramm and co-workers.[30]

Acknowledgment. We thank Dr. Forrest A. MacKellar and his associates for spectral determinations and microanalyses. We also thank Dr. S. Priebe for performing HPLC analysis on racemic and the pure enantiomers of 54.

Synthesis and Biological Evaluation of Substituted Benzenesulfonamides as Novel Potent Membrane-Bound Phospholipase A_2 Inhibitors

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A novel series of 4-[*N*-methyl-*N*-[(*E*)-3-[4-(methylsulfonyl)phenyl]-2-propenoyl]amino]benzenesulfonamides has been prepared and evaluated as membrane-bound phospholipase A_2 inhibitors. A structure–activity relationship study indicated that the optimum potency was realized with the *N*-(phenylalkyl)piperidine derivatives **3** and **4**. These compounds inhibited the liberation of arachidonic acid from the rabbit heart membrane fraction with IC_{30} values of 0.028 and 0.009 μM, respectively. Several compounds (**3**, **4**, and **28**), which proved to be potent inhibitors in vitro, significantly reduced the size of myocardial infarction in coronary occluded rats by iv administrations prior to the ligation. *N*-(1-Benzyl-4-piperidinyl)-4-[*N*-methyl-*N*-[(*E*)-3-[4-(methylsulfonyl)phenyl]-2-propenoyl]amino]-benzenesulfonamide (**3**, ER-3826), which showed the protective in vivo effects at doses higher than 0.3 mg/kg iv, was finally chosen as a leading candidate.

Phospholipase A_2 (PLA_2) is an enzyme that catalyses the hydrolysis of the fatty acid ester bond at the 2-position of membrane phospholipids to produce two potent inflammatory mediators, e.g., arachidonic acid (AA) and lysophospholipids.[1-3] This enzyme is usually classified into two species, extracellular PLA_2 and intracellular PLA_2. The former PLA_2 is found in the venoms of bees and snakes and mammalian pancreatic secretions and is well characterized both mechanistically and with regard to their primary sequence.[4] The ability to inhibit the extracellular PLA_2 has been the focus of several laboratories for the potential discovery of antiinflammatory agents.[1,5] In contrast, little is known about intracellular PLA_2', thereby the pharmacological consequences caused by the inhibition of this enzyme are not well-understood.[6]

[†] Eisai Co., Ltd.
[‡] University of Tsukuba.

(1) Lapetina, E. G. *Annu. Rep. Med. Chem.* **1984**, *19*, 213.
(2) Needleman, P.; Turk, J.; Jakschik, B. A.; Morrison, A. R.; Lefkowith, J. B. *Annu. Rev. Biochem.* **1986**, *55*, 69.
(3) (a) Hanahan, D. J. *Annu. Rev. Biochem.* **1986**, *55*, 483. (b) Snyder, F. *Annu. Rep. Med. Chem.* **1983**, *17*, 314.
(4) (a) Slotboom, A. J.; Verheij, H. M.; DeHaas, G. H. *Posopholipids New Biochemistry*; Hawthrone and Ansell, Eds.; Elsevier: Amsterdam, 1982; Vol. 4, p 359. (b) Volwerk, J. J.; DeHaas, G. H. *Lipid-Protein Interactions*; Jost, P. C., Griffith, O. H., Eds.; John Wiley and Sons: New York, 1982; p 69. (c) Velheij, H. M.; Slotboom, A. J.; DeHaas, G. H. *Rev. Physiol. Biochem. Pharmacol.* **1981**, *91*, 91. (d) Heinrikson, R. L.; Sakman, T. P.; Randolph, A. *Frontiers in Protein Chemistry*; Liu, T. Y., Mamiya, C., Yasunobu, K., Eds.; Elsevier, North Holland Biomedical Press: Amsterdam, 1980; p 297.

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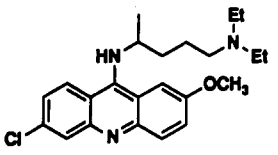
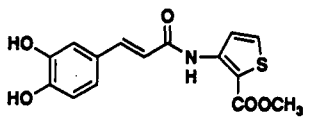
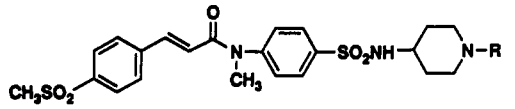
Recently, it has been suggested that the activation of intracellular membrane-bound PLA_2 during acute ischemia could be responsible for irreversible damage in several organs including the liver and the heart.[7] This hypothesis was supported by the observation that pretreatment of the tested animals with mepacrine (1), which is a nonspecific inhibitor of PLA_2,[8] prevented changes in myocardial contractility occurring in the nonischemic regions following coronary artery occlusion.[9] However, mepacrine (1) also exhibits cyclooxygenase-blocking activity.[8] This indicates that the biological effect of this drug may be related not only to PLA_2 inhibition but also to other enzymes in the AA cascade. Therefore, the relationship between the beneficial effect of the inhibition of intracellular membrane-bound PLA_2 and the prevention of ischemic myocardial injury is still unclear. In order to clarify the above pharmacological contribution of the inhibition of intracellular PLA_2 and to find new inhibitors useful for the protection of various ischemic diseases, we have searched for potent and specific membrane-bound PLA_2 inhibitors.



mepacrine 1

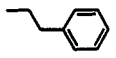


2



3: R =

4: R =



In the early stage of our study, compound **2**,[10] which was discovered by Beecham's researchers as an extracellular PLA_2 inhibitor, was found to weakly inhibit the liberation of AA from rabbit heart membrane fraction (32% at 100 μM). We were particularly interested in the unique structure of compound **2**, which includes a catechol and a thiophene–ester moiety in the molecule. We then prepared wide variants of compound **2** and evaluated their in vitro activity. Accordingly, novel 4-[*N*-[3-(4-substituted-phenyl)-2-propenoyl]amino]benzenesulfonamides were found to possess potent inhibitory activity, using rabbit heart membrane-bound PLA_2. Of these, *N*-(1-benzyl-4-piperidinyl)-4-[*N*-methyl-*N*-[(*E*)-3-[4-(methylsulfonyl)phenyl]-2-propenoyl]amino]benzenesulfonamide (**3**) and its 1-(2-phenylethyl)piperidine derivative **4** were the most potent inhibitors. The IC_{30} values of these compounds were 0.028 and 0.009 μM, respectively.

In this paper, we describe the preparation of the novel benzenesulfonamide derivatives and their biological evaluation using rabbit heart intracellular membrane-bound PLA_2. The effects of the candidate compounds **3**, **4**, and **28**, which proved to be potent inhibitors in vitro, on the prevention of acute myocardial infarction after coronary artery occlusion in rats are also described.

Chemistry

The synthetic sequence leading to the 4-[*N*-[3-(3,4-dihydroxyphenyl)-2-propenoyl]amino]benzenesulfonamides **9–18** is outlined in Scheme I. Sulfonyl chlorides **5a** and **5b**[11] were treated with amines **6**, and the protecting acyl groups were hydrolyzed to give 4-aminobenzenesulfonamides **7a–w**. Condensation of **7** with acid chloride **8**[10] and subsequent hydrolysis of the diacetate groups gave catechol derivatives **9–18**. Compounds **3**, **4**, and **20–37**, in which the catechol moiety was replaced with other polarizable substituents such as an imidazolyl-, nitro-, and sulfonylphenyl group, were prepared by a method similar to that used for the preparation of the catechol analogues as shown in Scheme II. Condensation of acid chlorides **19**[12] with sulfonamides **7**, and the following cleavage of an ester or ether bond with aqueous hydrochloric acid or boron tribromide, afforded target compounds **3**, **4**, and **20–37**.

Pharmacology

PLA_2 activity in rabbit heart membrane fraction was evaluated by a modified method of Waite et al.,[13] using an incubation of 1-palmitoyl-2-([^{14}C]arachidonyl)phosphatidyl choline as a substrate. Each compound was tested in triplicate at several different concentrations, and the radioactivity of the liberated ^{14}C-arachidonic acid (^{14}C-AA) was measured. The average value of inhibitory activity for each concentration was plotted to identify for each compound an IC_{30}, the concentration needed to inhibit 30% of the liberation of ^{14}C-AA by rabbit heart membrane fraction. When the IC_{30} value of the tested compound was less than 30.0 μM, the in vitro test was repeated at least three times to provide its quantitative concentration response profile. The in vitro results of standards, mepacrine (1) and compound **2**, and synthesized compounds in this study on PLA_2 inhibition are shown in Tables I–III.

In vivo effects of compounds, which proved to be active in vitro, on the protection of ischemic myocardial injury were evaluated by using an immunohistochemical technique and an image analyzer in coronary-ligated rats. In a previous paper, we reported that the immunohistochemical loss of myoglobin from the myocardial cells serves

(5) (a) Montasim, B. A. *Annu. Rep. Med. Chem.* **1987**, *22*, 223. (b) Ripka, W. C.; Sipio, W. J.; Blaney, J. M. *Lectures in Heterocyclic Chemistry IX*, 1987; p 95. (c) For review: Wilkerson, W. W. *Drugs Future* **1990**, *15*(2), 139.

(6) (a) Jimeno-Abendano, J.; Zahler, P. *Biochem. Biophys. Acta* **1979**, *573*, 266. (b) Nixon, J. S.; Wilkinson, S. E.; Davis, P.; Boxham, D. P. *Agents Actions* **1985**, *17*, 299. (c) Chang, J.; Blazek, E.; Lewis, J. *Agents Actions* **1985**, *17*, 296.

(7) (a) Needleman, P.; Marshall, G. R.; Sabel, B. E. *Circ. Res.* **1975**, *37*, 872. (b) Chien, K. R.; Abrams, J.; Serroni, A.; Martin, J. T.; Farber, J. L. *Biol. Chem.* **1978**, *253*, 4809. (c) Chien, K. R.; Pfau, R. G.; Farber, J. L. *Am. J. Pathol.* **1979**, *97*, 505. (d) Chien, K. R.; Reeves, J. P.; Buja, M.; Bonte, F.; Parkey, R.; Willerson, J. T. *Circ. Res.* **1981**, *48*, 711. (e) Sobel, B. E.; Corr, P. B. *Adv. Cardiol.* **1979**, *26*, 76.

(8) Blackwell, G. J. *Adv. Prostaglandin Thronboxane Res.* **1978**, *3*, 137.

(9) Meerson, I. Z.; Dosmagambetova, R. S. *Biull. Eksp. Biol. Med.* **1983**, *95*, 167.

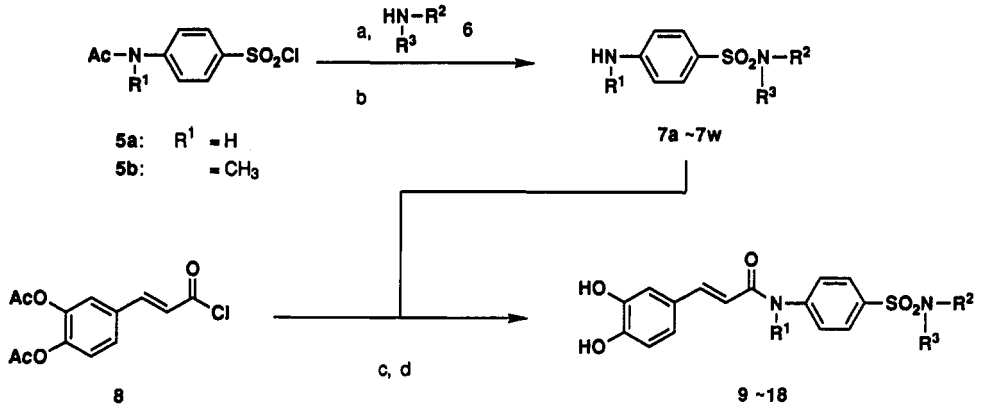
(10) Outred, D. J. European Patent, EP 277431, 1981.

(11) (a) Stojanovic, O. K.; Boncic-Caricic, G. A.; Dimitrijevic, D. M. *Glas. Hem. Drus.*, Beograd **1971**, *36*(9–10), 393; (b) *Chem. Abstr.* **1972**, *78*(1), 3902s.

(12) The acid chlorides **19a–f** were prepared from the corresponding acids by a similar procedure used for the preparation of **8**. **19a–f** were used in next reactions without further purification. (a) For **19a**: Sakakibar, M.; Matsui, M. *Agric. Biol. Chem.* **1979**, *43*, 117. (b) For **19b**: Gustafsson, B. B. R.; Nitenberg, T. *Acta. Pharm. Suec.* **1986**, *23*(4), 241. (c) For **19c**: Iizuka, K.; Akahane, K.; Kamijo, Y.; Monose, D.; Ajisawa, M.; Okaya, Y. Ger Offen. 2923815 1978. (d) For **19f**: Dani, V. R.; Nargund, K. S. *J. Karnatak Univ.* **1959**, *4*, 26; *Chem. Abstr.* **1961**, *55*(8), 27201d.

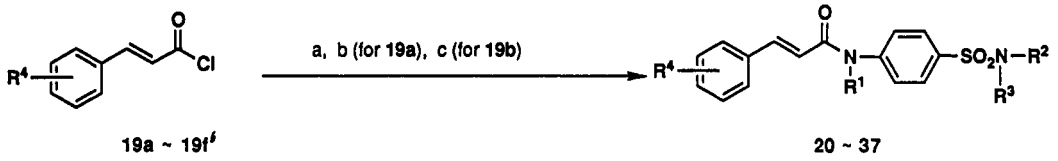
(13) Waite, M.; Scherphof, G. L.; Boshouweres, F. M. G.; VanDeenen, L. L. M. *J. Lipid Res.* **1969**, *10*, 411.

Scheme I. Preparation of 4-[*N*-[3-(3,4-Dihydroxyphenyl)-2-propenoyl]amino]benzenesulfonamides[a]



[a] (a) amine 6, CH_3COONa, ethanol, room temperature, 4 h; (b) 1 N NaOH, reflux, 2 h; (c) pyridine, CH_2Cl_2, 0 °C, 2 h; (d) THF–methanolic 2 N HCl, 60 °C, 20 min.

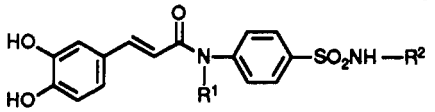
Scheme II. Preparation of Non-Catechol Derivatives 3, 4,and 20–37[a]

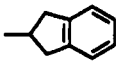
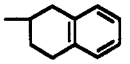
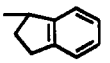


19a ~ 19f[b] 20 ~ 37

[a] (a) Sulfonamide 7, pyridine, CH_2Cl_2, 0 °C, 2 h; (b) THF–methanolic 2 N HCl, 60 °C, 20 min; (c) BBr_3, CH_2Cl_2, reflux, 3 h. [b] **19a** (R^4 = 4-OAc), **19b** (R^4 = 3-F and 4-OMe), **19c** [R^4 = 4-(1-imidazolyl)], **19d** (R^4 = 4-CN), **19e** (R^4 = 4-NO_2), **19f** (R^4 = 4-SO_2CH_3).

Table I. Physical Properties and Inhibitory Activities of 4-[*N*-3-(3,4-Dihydroxyphenyl)-2-propenoyl]amino]benzenesulfonamides 9–18



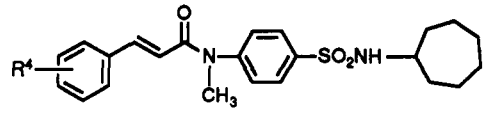
no.	R^1	R^2	amine 7 (% yield)[a]	formula[b]	mp, °C	solvent[c]	IC_{30} (μM)[d]	n[e]
				mepacrine (1)			14.9 ± 1.8% (100 μM)	4
2							31.6 ± 6.6% (100 μM)	4
9	H	H	(56)[f]	$C_{15}H_{14}N_2O_5S$	292–294	MeOH–H_2O	25.0 ● 5.00	3
10	CH_3	H	7a (53)	$C_{16}H_{16}N_2O_5S$	205–206	Et_2O–AcOEt	13.3 ± 2.52	3
11	CH_3	CH_3	7b (81)	$C_{17}H_{18}N_2O_5S$·0.1H_2O	180–182	Et_2O–AcOEt	44	1
12	CH_3	$CH(CH_3)_2$	7c (95)	$C_{19}H_{22}N_2O_5S$	197–199	MeOH–H_2O	30	1
13	CH_3		7d (66)	$C_{21}H_{24}N_2O_5S$	209–211	Et_2O–AcOEt	5.43 ● 2.35	3
14	CH_3		7e (89)	$C_{22}H_{26}N_2O_5S$	224–225	*i*-Pr_2O–AcOEt	1.45 ± 0.35	3
15	CH_3		7f (63)	$C_{23}H_{28}N_2O_5S$	207–210	Et_2O–AcOEt	1.75 ± 0.05	3
16	CH_3		7g (91)	$C_{25}H_{24}N_2O_5S$	201–203	Et_2O	4.64 ± 0.80	5
17	CH_3		7h (90)	$C_{26}H_{26}N_2O_5S$	277–280	*i*-Pr_2O–AcOEt	5.07 ± 1.22	3
18	CH_3		7i (86)	$C_{25}H_{24}N_2O_5S$	202–206	*i*-Pr_2O	6.10 ± 0.66	3

[a] Represents overall yield of purified material from condensation of acid chloride 8 with amine 7. [b] Analytical results were within ±0.4% of the theoretical values for C, H, and N. [c] Purified by recrystallization from the shown solvents. [d] Tested using rabbit heart membrane fraction and 1-palmitoyl-2-([^{14}C]arachidonyl)phosphatidyl choline as a substrate. Values are mean ● SEM. See the Experimental Section for assay protocol. [e] Number of experiments. Each compound was tested in triplicate at several different concentrations. [f] 4-Aminobenzenesulfonamide was used as an amine.

as an early end sensitive marker of irreversible cell damage after myocardial ischemia.[14] In this assay, the myoglobin-depleted area of the rat ventricular myocardium in the early phase of ischemia was measured as an index of myocardial infarction. Each compound was administered intravenously (iv) 15 min prior to the ligation, and the coronary artery was ligated for 3 h. Staining and morphometric analysis were carried out according to the method reported previously. The results of the in vivo tests

(14) (a) Nomoto, K.; Mori, N.; Miyamoto, J.; Shoji, T.; Nakamura, K. *Experimental Molecular Pathol.* **1989**, *51*, 231. (b) Nomoto, K.; Mori, N.; Shoji, T.; Nakamura, K. *Exp. Mol. Pathol.* **1987**, *47*, 390.

Table II. Physical Properties and Inhibitory Activities of Non-Catechol Derivatives **20–25**



no.	R^4	halide **19** (% yield)[a]	formula[b]	mp, °C	solvent[c]	IC_{30} (μM)[d]	n[e]
20	4-OH	**19a** (71)	$C_{23}H_{28}N_2O_4S$	187–189	*i*-Pr_2O–AcOEt	1.24 ± 0.64	3
21	3-F, 4-OH	**19b** (55)	$C_{23}H_{27}FN_2O_4S$	190–191	*i*-Pr_2O–AcOEt	5.33 ± 0.67	3
22	4-(1-imidazolyl)	**19c** (79)	$C_{26}H_{30}N_4O_3S$	173–174	Et_2O–AcOEt	0.45 ± 0.11	3
23	4-CN	**19d** (65)	$C_{24}H_{27}N_3O_5S$	170–171	Et_2O–AcOEt	0.20 ± 0.05	3
24	4-NO_2	**19e** (86)	$C_{23}H_{27}N_3O_5S$	187–188	*i*-Pr_2O–AcOEt	0.19 ± 0.16	3
25	4-SO_2CH_3	**19f** (78)	$C_{24}H_{30}N_2O_5S$	146–148	MeOH	0.016 ± 0.003	3

[a] Represents overall yield of purified material from condensation of acid chloride **19** with amine **7f**. [b] Analytical results were within ±0.4% of the theoretical values for C, H, and N. [c] Purified by recrystallization from the shown solvents. [d] Tested using rabbit heart membrane fraction and 1-palmitoyl-2-([^{14}C]arachidonyl)phosphatidyl choline as a substrate. Values are mean ± SEM. See the Experimental Section for assay protocol. [e] Number of experiments. Each compound was tested in triplicate at several different concentrations.

are summarized in Table IV.

Discussion

The IC_{30} values of mepacrine (**1**) and **2** on rabbit heart membrane fraction were over and about 100 μM, respectively. We then focused on modifying the structure of compound **2** and replaced the thiophene–ester moiety with other aryl groups such as benzene esters or benzenesulfonamides in order to enhance the inhibitory activity. In the preliminary evaluations, the activity was increased about 4-fold by the introduction of N-nonsubstituted benzenesulfonamide (compound **9**, IC_{30} = 25 μM), and *N*-(methylamino)benzenesulfonamide **10** also showed much stronger activity (IC_{30} = 13 μM) than that of compounds **1** and **2**.

Then, the variants of compound **10** at the substituents (R^2 and R^3) on the nitrogen atom of the sulfonamide were prepared and evaluated (Table I). Of these, the introduction of hydrophobic substituents such as cycloalkyl and benzene-fused cycloalkyl groups significantly increased inhibitory activity (compounds **13–18**, IC_{30} = 1–5 μM).

Although we obtained satisfactory results on the in vitro tests in compounds **13–18** as mentioned above, their metabolic instability and low water solubility were thought to be the obstacles in evaluating in vivo experiments. The former problem may be mainly attributed to the presence of the catechol moiety, and the latter to the absence of an ionizable functionality. Therefore, to increase the metabolic stability and water solubility of the compounds, non-catechol derivatives, tertiary aminoalkylbenzenesulfonamides, and their alkylpyridininyl derivatives were designed and prepared. The results are shown in Tables II and III.

Among non-catechol derivatives, 4-hydroxy derivative **20** showed a comparable activity to that of the corresponding catechol analogue **15**, and 3-fluoro-4-hydroxy derivative **21** showed slightly a less potent activity. It was noticed that the activity of 4-imidazol-1-yl) derivative **22**, exhibiting 0.45 μM for the IC_{30} value, was much superior to the above non-catechol compounds. Furthermore, the introduction of more electron withdrawing substituents such as a cyano, nitro, and sulfonyl groups to the 4-position of the benzene ring resulted in great improvements in inhibitory activity (**23**, **24**, and **25**). In particular, the activity of methylsulfonyl derivative **25** was 0.016 μM, which is almost 100 times more potent than that of the corresponding catechol derivative **15**.

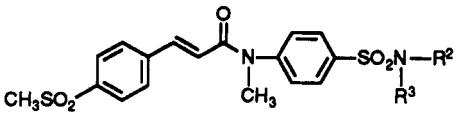
2-Pyridinylalkyl derivatives **26** and **27** and the 6-methyl-2-pyridyl analogue **28** maintained the activity to a certain extent (Table III). The introduction of a methyl group as the substituent R^3 did not affect the activity (**28**/**29**). 1-Piperidinyl and 1-homopiperidinyl analogues **30–33** also showed high activity comparable to that of 2-pyridinylalkyl derivatives. The high activity was also maintained by the introduction of *N*-substituted piperidin-4-yl and homopiperidin-3-yl groups as R^2 (**3**, **4**, **35–37**). Interestingly, the potency of inhibitory activity seems to correlate with the lipophilicity or steric bulkiness of the substituents on the nitrogen atom of the cyclic amines. Thus, the IC_{30} value of *N*-methylpiperidin-4-yl derivative **34** was 1.00 μM, a relatively higher concentration, whereas more lipophilic and bulky compounds such as benzyl and phenylethyl derivatives **3** and **4** had much more potent IC_{30} values of 0.028 and 0.009 μM, respectively. *N*-Substituted homopiperidin-3-yl derivatives **36** and **37** also showed potent activity similar to that of piperidine analogue **3**. The activity of compound **4** was the best in the compounds synthesized in this study. The IC_{30} value of **4** was about 1300 times stronger than that of compound **10**, which was one of the initial lead compounds in this study.

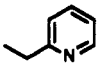
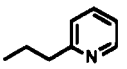
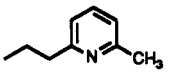
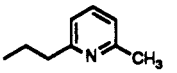
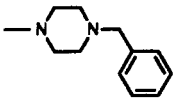
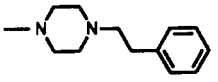
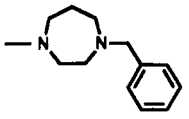
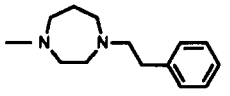
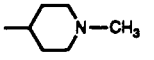
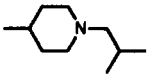
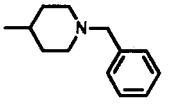
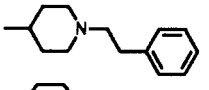
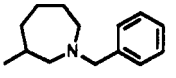
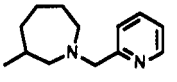
The metabolic stability of compounds in plasma after iv administration to rats was significantly improved in non-catechol derivatives. The catechol derivative **14** had a very short half-live of β elimination phase ($t_{1/2\beta}$) of 11 min. However, the half-lives were prolonged to more than 30 min in non-catechol analogues, which had a 4-(1-imidazolyl) or methylsulfonyl group as substituent R^4 (**22**; 30 min, **25**; 65 min). Water-soluble compound **3** also has satisfactory metabolic stability ($t_{1/2\beta}$ = 43 min).

Table IV shows the effects of standards, mepacrine (**1**) and varapamil, and several selected compounds, **3**, **4**, and **28**, on the protection of ischemic myocardial injury after coronary artery occlusion in rats. Mepacrine protected the progression of myocardial infarct size at 75 mg/Kg sc administration. Verapamil, a popular calcium entry blocker that has been reported to be effective for acute myocardial infarction in several animal models[15] was also effective at 1.0 mg/kg iv (inhibition rate = 19%). Compared with these standards, compounds **3** and **4** significantly inhibited the infarct size at only 0.3 and 1.0 mg/Kg iv administrations, respectively (inhibition rate = 21% and 23%). Furthermore, the selected compounds, **3**, **4**, and **28**, had little effects on the hemodynamics of the cardiovascular system such as heart rate (HR), mean atrial pressure (MAP), and the first derivative of left ventricular pressure (LV dp/dt) in anesthetized dogs (unpublished data).[16]

(15) (a) Reimer, K. A.; Lowe, J. E.; Jennings, R. B. *Circulation* **1977**, *55*(4), 581. (b) Kloner, R. A.; DeBoer, L. W. V.; Carlson, N.; Braunwald, E. *Exp. Mol. Pathol.* **1982**, *36*, 277.

Table III. Physical Properties and Inhibitory Activities of 4-[*N*-[3-[4-Methylsulfonyl)phenyl]-2-propenoyl]amino]benzenesulfonamides **3**, **4**, and **26–37**



no.	R^3	R^2	amine 7 (% yield)[a]	formula[b]	mp, °C	solvent[c]	IC_{30}, (μM)[d]	n[e]
26	H		**7j** (91)	$C_{23}H_{23}N_3O_5S_2$·HCl	125–128	CH_2Cl_2–EtOH	0.26 ± 0.15	3
27	H		**7k** (83)	$C_{24}H_{25}N_3O_5S_2$·0.5H_2O	143–144	Et_2O–EtOH	0.21 ± 0.03	3
28	H		**7l** (79)	$C_{25}H_{27}N_3O_5S_2$·HCl·0.2H_2O	169–175	AcOEt	0.066 ± 0.030	5
29	CH_3		**7m** (51)	$C_{26}H_{29}N_3O_5S_2$·HCl	164–167	AcOEt–EtOH	0.065 ± 0.029	4
30			**7n** (74)	$C_{28}H_{31}N_3O_5S_2$·HCl·0.5H_2O	194–196	CH_2Cl_2	0.22 ± 0.18	3
31			**7o** (87)	$C_{29}H_{33}N_3O_5S_2$	193–194	AcOEt	0.063 ± 0.018	3
32			**7p** (89)	$C_{29}H_{33}N_3O_5S_2$·HCl	196–199	AcOEt	0.064 ± 0.008	3
33			**7q** (77)	$C_{30}H_{35}N_3O_5S_2$·HCl	203–207	AcOEt–EtOH	0.025 ± 0.008	3
34	H		**7r** (65)	$C_{23}H_{29}N_3O_5S_2$	122–125	AcOEt	1.00 ± 0.36	3
35	H		**7s** (39)	$C_{26}H_{35}N_3O_5S_2$·0.4H_2O	147–148	Et_2O–AcOEt	0.051 ± 0.15	3
3	H		**7t** (70)	$C_{29}H_{33}N_3O_5S_2$	189–190	Et_2O–AcOEt	0.028 ± 0.012	7
4	H		**7u** (74)	$C_{30}H_{35}N_3O_5S_2$	173–176	Et_2O–AcOEt	0.009 ± 0.004	6
36	H		**7v** (44)	$C_{30}H_{35}N_3O_5S_2$[f]	88–92 amorphous	*i*-Pr_2O	0.025 ± 0.009	3
37	H		**7w** (22)	$C_{29}H_{34}N_4O_5S_2$·2HCl[g]	145–148	Et_2O–AcOEt	0.049 ± 0.017	3

[a] Represents overall yield of purified material from condensation of acid chloride **19f** with amine **7**. [b] Analytical results were within ±0.4% of the theoretical values for C, H, and N unless otherwise noted. [c] Purified by recrystallization from the shown solvents. [d] Tested using rabbit heart membrane fraction and 1-palmitoyl-2-([^{14}C]arachidonyl)phosphatidyl choline as the substrate. Values are mean ± SEM. See the Experimental Section for assay protocol. [e] Number of experiments. Each compound was tested in triplicate at several different concentrations. [f] Calcd for C 61.96, found 61.41. High mass: calcd 582.2097, found 582.2102. [g] Calcd for C 53.12, found 52.70. High mass: calcd 582.1970, found 582.1964.

Verapamil, on the other hand, did affect these hemodynamics. In in vitro tests, leading candidates, compounds **3** and **4**, did not show cyclooxygenase and lipoxygenase inhibitory activity in rat platelet (i.e., $IC_{50} > 10^{-4}$ M) or calcium channel-blocking effect in rat aorta (i.e., $ED_{50} > 10^{-4}$ M, KCl concentration; 50 mM). Therefore, it would be reasonable to conclude that the protection observed with those compounds against myocardial ischemic injury caused by coronary artery occlusion in rats is attributable to their inhibition of heart membrane-bound PLA_2.

Conclusion

We have reported here on the synthesis and pharmacological evaluation of novel 4-[*N*-methyl-*N*-[(*E*)-3-[4-(methylsulfonyl)phenyl]-2-propenoyl]amino]benzene-

(16) Recently, Maroko et al. also reported the effects of a PLA_2 inhibitor on experimental infarct size, left ventricular hemodynamics, and regional miocardial blood flow: Zalewsk, A.; Goldberg, S.; Maroko, P. R. *Int. J. Cardiol.* **1988**, *21*, 247.

Table IV. Comparison of Infarct Size in Drug-Treated Rats Subjected to 3-h Occlusion[a]

compd	dose (mg/kg)	route[b]	inhibtn rate (%)[c]	n[d]
mepacrine (1)	75.0	sc	37.4 ± 4.3[e]	10
verapamil	0.1	iv	0.7 ± 14.7	7
	0.3	iv	0.1 ± 6.9	7
	1.0	iv	19.2 ± 4.6[e]	23
3	0.1	iv	1.6 ± 7.7	5
	0.3	iv	20.9 ± 4.1[e]	24
	1.0	iv	22.1 ± 3.6[e]	23
4	0.1	iv	7.0 ± 9.7	6
	0.3	iv	18.7 ± 6.9	14
	1.0	iv	22.5 ± 5.3[e]	15
28	1.0	iv	5.6 ± 5.7	13
	10.0	iv	21.9 ± 8.0[e]	14

[a] Each compound was administered 15 min prior to the ligation. [b] Administration route. [c] Inhibition rate of infarct size (myoglobin depleted area). Each value was mean ± SEM. [d] Number of animals. [e] $P < 0.05$, vs infarct size of untreated animals.

sulfonamides as membrane-bound phospholipase A_2 (PLA_2) inhibitors. The structure–activity relationship studies revealed that the electron-withdrawing character of substituents R^4 at the 4 (para) position of the phenyl ring and the lipophilicity or steric effect of the substituents R^2 on the nitrogen atom of the sulfonamide group were important for enhancing the inhibitory activity. Compounds **3** and **4** were the most potent inhibitors of membrane-bound PLA_2 (IC_{30} values $<10^{-8}$ M). In in vivo experiments, compounds **3** and **4** significantly protected the ischemic damage in acute myocardial infarction in rats by 0.3 and 1.0 mg/kg iv administrations, respectively. Although the contributions of heart membrane-bound PLA_2 to ischemic myocardial injury are unclear even now, the present results suggest that membrane-bound PLA_2 inhibitors may be clinically effective in the protection of acute myocardial infarction. Further pharmacological and medicinal investigation on the candidate compound **3** are in progress, and the results will be reported in forthcoming publications.

Experimental Section

Materials and Methods. Chemistry. Solvents were of reagent grade. The purity of each product was checked by thin layer chromatography (TLC) on silica gel plates (Kieselgel 60 F_{254}, thickness 0.25 mm). Column chromatography was performed on silica gel (Merck, particle size 0.063–0.200 mm for normal chromatography, middle particle size 15 μm for flash chromatography). All melting points (mp) were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on JEOL-JNM-FX90Q (90 MHz) and JEOL-JNM-FX400 (400 MHz) instruments. Chemical shifts are reported in δ units, using tetramethylsilane as an internal standard. Mass spectra were obtained on a JEOL-HX100 mass spectrometer and are tabulated as m/e. Elemental analyses were performed at the Analytical Chemistry Section of Eisai Tsukuba Research Laboratories.

Preparation of Substituted 4-Aminobenzenesulfonamides 8. General Procedure. 4-(*N*-Acetyl-*N*-methylamino)benzenesulfonyl chloride (**5b**) was prepared by a similar method to that described by Stjanovic et al.:[11] yield 42%; mp 139–140 °C (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 8.04 (2 H, d, J = 9.0 Hz), 7.43 (2 H, d, J = 9.0 Hz), 3.36 (3 H, s), 2.08 (3 H, s). To a suspension of amine **6** (100 mmol) and sodium acetate (250 mmol) in ethanol (200 mL) was added sulfonyl chloride **5b** (91 mmol) at 0 °C, and the mixture was stirred at room temperature for 4 h. Water was added to the mixture, and the resulting solution was extracted with ethyl acetate or dichloromethane. The extract was washed with water and brine, dried ($MgSO_4$), and evaporated. The residual sulfonamide was dissolved in a solution of ethanol (100 mL) and aqueous 1 N NaOH solution (100 mL), and the mixture was refluxed overnight. The reaction mixture was neutralized with aqueous HCl solution, and the resulting mixture was extracted with ethyl acetate or dichloromethane. The extract was washed with water and brine, dried ($MgSO_4$), and evaporated. The residual solid was purified by recrystallization or silica gel column chromatography to give sulfonamides **7a–r,v,w**.

4-(Methylamino)benzenesulfonamide (7a): overall 79%; mp 166–167 °C; NMR (400 MHz, DMSO-d_6) δ 7.51 (2 H, d, J = 8.8 Hz), 6.91 (2 H, s), 6.57 (2 H, d, J = 8.8 Hz), 6.37 (1 H, br q, J = 5.0 Hz), 2.71 (3 H, s).

***N*-Methyl-4-(methylamino)benzenesulfonamide hydrochloride (7b)**: 81%; mp 143–148 °C; NMR (90 MHz, DMSO-d_6) δ 7.56 (2 H, br, D_2O exchange), 7.50 (2 H, d, J = 8.4 Hz), 6.75 (2 H, d, J = 8.4 Hz), 2.72 (3 H, s), 2.32 (3 H, s).

***N*-Isopropyl-4-(methylamino)benzenesulfonamide hydrochloride (7c)**: 70%; mp 120–128 °C; NMR (400 MHz, DMSO-d_6) δ 7.49 (2 H, d, J = 8.8 Hz), 7.07 (1 H, br), 6.61 (2 H, d, J = 8.8 Hz), 3.10 (1 H, m), 2.71 (6 H, s), 0.96 (6 H, d, J = 7.0 Hz).

***N*-Cyclopentyl-4-(methylamino)benzenesulfonamide hydrochloride (7d)**: 51%; mp 143–147 °C; NMR (400 MHz, DMSO-d_6) δ 7.51 (2 H, d, J = 8.8 Hz), 6.68 (2 H, d, J = 8.8 Hz), 3.30 (1 H, m), 2.73 (3 H, s), 1.53 (2 H, m), 1.32 (2 H, m).

***N*-Cyclohexyl-4-(methylamino)benzenesulfonamide hydrochloride (7e)**: 70%; mp 130–131 °C; NMR (400 MHz, $CDCl_3$/DMSO-d_6) δ 8.73 (2 H, br s), 7.91 (2 H, d, J = 8.5 Hz), 7.56 (2 H, d, J = 8.5 Hz), 2.99 (3 H, s), 1.67 (4 H, m), 1.52 (1 H, br d, J = 12.1 Hz), 1.18 (4 H, m), 1.10 (1 H, m).

***N*-Cycloheptyl-4-(methylamino)benzenesulfonamide (7f)**: 42%; mp 130–135 °C; NMR (400 MHz, DMSO-d_6) δ 7.51 (2 H, d, J = 8.8 Hz), 6.68 (2 H, d, J = 8.8 Hz), 3.05 (1 H, m), 2.73 (3 H, s), 1.60–1.50 (12 H, m), 1.25 (2 H, m).

***N*-(2-Indanyl)-4-(methylamino)benzenesulfonamide (7g)**: 82%; mp 136–137 °C; NMR (90 MHz, DMSO-d_6) δ 7.85 (2 H, d, J = 8.8 Hz), 7.53 (2 H, d, J = 8.8 Hz), 7.08 (4 H, s), 3.90 (1 H, m), 3.24 (3 H, s), 3.12–2.54 (4 H, m).

***N*-[2-(1,2,3,4-Tetrahydronaphthyl)]-4-(methylamino)benzenesulfonamide (7h)**: 51%; mp 110–113 °C; NMR (400 MHz, DMSO-d_6) δ 7.54 (2 H, d, J = 8.8 Hz), 7.32 (1 H, d, J = 6.2 Hz), 7.50–6.99 (3 H, m), 6.95 (1 H, m), 6.60 (2 H, d, J = 8.8 Hz), 6.49 (1 H, br q, J = 5.0 Hz), 3.20 (1 H, m), 2.83–2.54 (4 H, m), 2.73 (3 H, d, J = 5.0 Hz), 1.80 (1 H, m), 1.56 (1 H, m).

***N*-(1-Indanyl)-4-(methylamino)benzenesulfonamide (7i)**: 49%; mp 117–119 °C; NMR (400 MHz, DMSO-d_6) δ 7.65 (1 H, d, J = 8.7 Hz), 7.57 (2 H, d, J = 8.8 Hz), 7.21–7.09 (4 H, m), 6.62 (2 H, d, J = 8.8 Hz), 6.51 (1 H, br), 4.56 (1 H, q like, J = 7.8 Hz), 2.79 (1 H, m), 2.74 (3 H, d, J = 4.2 Hz), 2.62 (1 H, m), 2.01 (1 H, m), 1.60 (1 H, m).

***N*-(2-Pyridylmethyl)-4-(methylamino)benzenesulfonamide dihydrochloride (7j)**: 90%; mp 196–201 °C; NMR (400 MHz, DMSO-d_6) δ 8.78 (2 H, d, J = 5.5 Hz), 8.49 (1 H, t, J = 7.9 Hz), 8.30 (1 H, br), 7.98 (1 H, d, J = 8.1 Hz), 7.90 (1 H, t, J = 6.5 Hz), 7.52 (2 H, d, J = 8.8 Hz), 6.60 (2 H, d, J = 8.8 Hz), 4.39 (2 H, s), 2.71 (3 H, s).

***N*-[2-(2-Pyridyl)ethyl]-4-(methylamino)benzenesulfonamide (7k)**: 53%; mp 99–100 °C; NMR (400 MHz, DMSO-d_6) δ 8.44 (1 H, ddd, J = 4.8, 1.8, 1.2 Hz), 7.67 (1 H, td, J = 7.6, 1.8 Hz), 7.47 (2 H, d, J = 8.8 Hz), 7.21 (2 H, m), 6.59 (2 H, d, J = 8.8 Hz), 6.49 (1 H, br q, J = 4.9 Hz), 3.00 (2 H, q like, J = 7.0 Hz), 2.80 (2 H, t, J = 7.5 Hz), 2.71 (3 H, d, J = 4.9 Hz).

***N*-[2-(6-Methyl-2-pyridyl)ethyl]-4-(methylamino)benzenesulfonamide dihydrochloride (7l)**: 93%; mp 170–172 °C; NMR (400 MHz, DMSO-d_6) δ 8.32 (1 H, br t, J = 8.0 Hz), 7.71 (1 H, d, J = 8.0 Hz), 7.65 (1 H, d, J = 8.0 Hz), 7.42 (2 H, d, J = 8.8 Hz), 6.57 (2 H, d, J = 8.8 Hz), 3.13 (4 H, br s), 2.72 (3 H, s), 2.71 (3 H, d, J = 5.2 Hz).

***N*-Methyl-*N*-[2-(6-methyl-2-pyridyl)ethyl]-4-(methylamino)benzenesulfonamide (7m)**: 83%; amorphous; NMR (400 MHz, $CDCl_3$) δ 7.56 (1 H, br t, J = 8.8 Hz), 7.49 (1 H, t, J = 8.0 Hz), 7.03 (1 H, d, J = 8.0 Hz), 6.98 (1 H, d, J = 8.0 Hz), 6.57 (2 H, d, J = 8.8 Hz), 4.28 (1 H, br q, J = 5.2 Hz), 3.36 (2 H, t, J = 7.2 Hz), 2.99 (2 H, t, J = 7.2 Hz), 2.87 (3 H, d, J = 5.2 Hz), 2.69 (3 H, s), 2.50 (3 H, s).

1-Benzyl-4-[[4-(methylamino)phenyl]sulfonyl]piperazine (7n): 79%; mp 146–147 °C; NMR (90 MHz, $CDCl_3$) δ 7.50 (2 H, d, J = 9.0 Hz), 7.21 (5 H, s), 6.55 (2 H, d, J = 9.0 Hz), 4.25 (1 H, br q, J = 5.8 Hz), 3.48 (2 H, s), 2.98 (4 H, m), 2.88 (3 H, d, J = 5.8 Hz), 2.54 (4 H, m).

1-[[4-(Methylamino)phenyl]sulfonyl]-4-[2-phenylethyl]-piperazine (7o): 66%; mp 203–204 °C; NMR (400 MHz, $CDCl_3$/DMSO-d_6) δ 7.48 (2 H, d, J = 8.9 Hz), 7.25 (2 H, m), 7.16 (3 H, m), 6.61 (2 H, d, J = 8.9 Hz), 5.60 (1 H, br q, J = 5.0 Hz), 2.97 (2 H, m), 2.96 (4 H, s), 2.84 (3 H, d, J = 5.0 Hz), 2.72 (2 H, m), 2.59 (4 H, m).

1-Benzyl-4-[[4-(methylamino)phenyl]sulfonyl]homopiperazine (7p): 39%; mp 140–141 °C; NMR (400 MHz, $CDCl_3$) δ 7.57 (2 H, d, J = 8.8 Hz), 7.28 (5 H, m), 6.58 (2 H, d, J = 8.8 Hz), 4.24 (1 H, br q, J = 5.1 Hz), 3.60 (2 H, s), 3.34 (4 H, m), 2.88 (3 H, d, J = 5.1 Hz), 2.66 (4 H, m), 1.80 (2 H, quint, J = 5.9 Hz).

1-[[4-(Methylamino)phenyl]sulfonyl]-4-(2-phenylethyl)-homopiperazine (7q): 87%; amorphous; NMR (400 MHz, $CDCl_3$) δ 7.58 (2 H, d, J = 8.9 Hz), 7.26 (2 H, m), 7.17 (3 H, m), 6.58 (2 H, d, J = 8.9 Hz), 4.23 (1 H, br q, J = 5.1 Hz), 3.34 (4 H, m), 2.88 (3 H, d, J = 5.1 Hz), 2.75 (4 H, m), 2.73 (4 H, s), 1.83 (2 H, quint, J = 6.0 Hz).

***N*-(1-Methyl-4-piperidinyl)-4-(methylamino)benzenesulfonamide (7r)**: 63%; amorphous; NMR (400 MHz, $CDCl_3$/DMSO-d_6) δ 7.42 (2 H, d, J = 9.0 Hz), 6.38 (2 H, d, J = 9.0 Hz), 5.92 (1 H, br d, J = 7.0 Hz), 4.94 (1 H, br q, J = 5.0 Hz), 2.78 (1 H, m), 2.64 (3 H, d, J = 5.0 Hz), 2.48 (2 H, br d, J = 11.7 Hz), 2.01 (3 H, s), 1.76 (2 H, br t, J = 11.7 Hz), 1.52 (2 H, m), 1.30 (2 H, m).

***N*-(1-Benzyl-4-piperidinyl)-4-(methylamino)benzenesulfonamide Dihydrochloride (7t)**. To a suspension of sulfonyl chloride **5b** (4.00 g, 16.2 mmol) and sodium acetate (2.65 g) in ethanol (40 mL) was added 4-amino-1-benzylpiperidine (3.63 mL, 17.8 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was extracted with ethyl acetate and the organic layer was washed with water and brine. After drying ($MgSO_4$), the solution was concentrated to afford crude *N*-(1-benzyl-4-piperidinyl)-4-(*N*-acetyl-*N*-methylamino)benzenesulfonamide as a syrup. The syrup was dissolved into an aqueous 4 N HCl solution and the mixture was refluxed for 3 h. The mixture was concentrated and the residual solid was recrystallized from ethyl acetate to give aniline **7t** as white crystals (5.80 g, 80% overall): highly hygroscopic, mp indistinct; NMR (400 MHz, DMSO-d_6) δ 7.60 (1 H, m), 7.54 (2 H, m), 7.50 (2 H, d, J = 8.8 Hz), 7.44 (1 H, m), 7.42 (2 H, m), 6.60 (2 H, d, J = 8.8 Hz), 4.13 (2 H, s), 3.18 (2 H, m), 3.08 (2 H, m), 2.87 (2 H, m), 2.71 (3 H, s), 1.73 (4 H, m).

***N*-[1-(2-Phenylethyl)-4-piperidinyl]-4-(methylamino)-benzenesulfonamide Dihydrochloride (7u)**. To a solution of *N*-(1-benzyl-4-piperidinyl)-4-(*N*-acetyl-*N*-methylamino)-benzenesulfonamide, which was prepared from sulfonyl chloride (20.0 g, 80.8 mmol) and 4-amino-1-benzylpiperidine (18.16 mL, 88.9 mmol) by the same procedure as described above, in ethanol (200 mL) were added acetic acid (10.0 mL) and Pd/C (2.00 g, 10% wet). The reaction mixture was vigorously stirred under a hydrogen atmosphere (1 atm) at 50 °C for 5 h. The catalyst was filtered off and the filtrate was concentrated to afford *N*-(4-piperidinyl)-4-(*N*-acetyl-*N*-methylamino)benzenesulfonamide acetate as white crystals (29.7 g, 100%): mp 177–184 °C; NMR (400 MHz, DMSO-d_6) δ 7.84 (2 H, d, J = 8.8 Hz), 7.55 (2 H, d, J = 8.8 Hz), 3.22 (3 H, s), 3.11 (1 H, m), 2.90 (2 H, m), 2.47 (2 H, m), 1.90 (3 H, br s), 1.85 (3 H, s), 1.57 (2 H, m), 1.32 (2 H, m). To a suspension of this amine (3.71 g, 10.0 mmol), sodium bicarbonate (2.52 g), and potassium iodide (3.32 g) in dimethylformamide (50 mL) was added (2-bromoethyl)benzene (1.49 mL, 11.0 mmol). The mixture was stirred at 70 °C for 3 h, poured into water, and extracted with ethyl acetate. The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated to afford crude *N*-[1-(2-phenylethyl)-4-piperidinyl]-4-(*N*-acetyl-*N*-methylamino)benzenesulfonamide as an oil (3.19 g, 77%). This oil was dissolved into 1 N NaOH (50 mL), and the mixture was refluxed for 2 h. The solution was neutralized by an aqueous HCl solution and extracted with ethyl acetate. The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated. The residual oil was converted into its dihydrochloride salt by ethanolic HCl solution, and crystallization of the salt from ethanol–ethyl acetate gave aniline **7u** as white crystals (2.55 g, 74%): highly hygroscopic, mp indistinct; NMR (400 MHz, DMSO-d_6) δ 7.55 (2 H, d, J = 8.8 Hz), 7.31 (3 H, m), 7.22 (2 H, m), 6.67 (2 H, d, J = 8.8 Hz), 3.45–3.00 (6 H, m), 2.92 (2 H, m), 2.72 (3 H, s), 1.75 (4 H, m).

***N*-(1-Isopropyl-4-piperidinyl)-4-(methylamino)benzenesulfonamide (7s)**: isopropyl bromide was used as a coupling reagent, overall 30%; mp 145–146 °C; NMR (400 MHz, $CDCl_3$/DMSO-d_6) δ 7.49 (2 H, d, J = 8.8 Hz), 7.20 (1 H, br d, J = 7.0 Hz), 6.58 (2 H, d, J = 8.8 Hz), 6.47 (1 H, br q, J = 4.7 Hz), 2.78 (1 H, m), 2.71 (3 H, d, J = 4.7 Hz), 2.62 (2 H, m), 1.92 (2 H, m), 1.80–1.60 (2 H, m), 1.50 (2 H, m), 1.32 (2 H, m), 0.79 (6 H, d, J = 6.4 Hz).

***N*-(1-Benzyl-3-homopiperidinyl)-4-(methylamino)-benzenesulfonamide (7v)**: 62%; amorphous; NMR (90 MHz, $CDCl_3$) δ 7.39 (2 H, d, J = 8.8 Hz), 7.31 (5 H, s), 6.44 (2 H, d, J = 8.8 Hz), 3.65–3.40 (3 H, m), 3.50–3.00 (2 H, m), 2.83 (3 H, br s), 2.70–2.20 (2 H, m), 2.00–1.05 (6 H, m).

***N*-[1-(2-Pyridylmethyl)-3-homopiperidinyl]-4-(methylamino)benzenesulfonamide (7w)**: 90%; amorphous; NMR (400 MHz, DMSO-d_6) δ 7.43 (2 H, d, J = 8.8 Hz), 7.36–7.27 (3 H, m), 7.21 (1 H, t like, J = 6.6 Hz), 6.58 (2 H, d, J = 8.8 Hz), 6.55 (1 H, m), 3.72 (1 H, d, J = 16.0 Hz), 3.68 (1 H, d, J = 16.0 Hz), 3.38 (1 H, m), 3.33 (2 H, s), 3.15 (1 H, m), 2.98 (1 H, m), 2.75 (1 H, m), 2.71 (3 H, d, J = 4.9 Hz), 2.64 (1 H, m), 1.77 (1 H, m), 1.64 (2 H, m), 1.52 (1 H, m), 1.31 (1 H, m).

Method A. *N*-(2-Indanyl)-4-[*N*-methyl-*N*-[(*E*)-3-(3,4-dihydroxyphenyl)-2-propenoyl]amino]benzenesulfonamide (16). To a solution of anilide **7g** (5.00 g, 16.6 mmol) in pyridine (50 mL) was added dropwise a solution of 3,4-diacetoxycinnamoyl chloride[10] (5.61 g, 19.9 mmol) in dichloromethane (50 mL) at 0 °C, and the mixture was stirred at room temperature for 2 h. Water was added to it and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with an aqueous 1 N HCl, water, an aqueous sodium bicarbonate solution, and brine. After drying over $MgSO_4$, the solution was concentrated to afford a solid, which was recrystallized from ethyl acetate–isopropyl ether to give *N*-(2-indanyl)-4-[*N*-methyl-*N*-[(*E*)-3-(3,4-diacetoxyphenyl)-2-propenoyl]amino]benzenesulfonamide as white crystals (8.90 g, 100%): mp 152–153 °C; NMR (400 MHz, DMSO-d_6) δ 8.11 (1 H, d, J = 7.2 Hz), 7.91 (2 H, d, J = 8.8 Hz), 7.58 (2 H, d, J = 8.8 Hz), 7.58–7.42 (3 H, m), 7.20 (1 H, d, J = 8.4 Hz), 7.11 (4 H, s), 6.61 (1 H, br d, J = 15.4 Hz), 3.94 (1 H, m), 3.38 (3 H, s), 2.98 (2 H, dd, J = 16.0, 8.0 Hz), 2.75 (2 H, dd, J = 16.0, 8.0 Hz), 2.26 (3 H, s), 2.18 (3 H, s); MS m/e (FAB) 549 (MH^+), 154 (base). Anal. ($C_{29}H_{28}N_2O_7S$), C, H, N.

To a suspension of this diacetate (8.90 g, 16.6 mmol) in methanol (80 mL) and tetrahydrofuran (80 mL) was added concentrated HCl (30 mL), and the mixture was stirred at 60 °C for 20 min. The mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated. The resulting solid was recrystallized from ether to give catecohol **16** as white crystals (7.17 g, 93%): mp 201–203 °C; NMR (90 MHz, DMSO-d_6) δ 9.20, 8.00 (each 1 H, br, D_2O exchange), 7.92 (2 H, d, J = 8.4 Hz), 7.55 (2 H, d, J = 8.4 Hz), 7.40 (1 H, d, J = 15.4 Hz), 7.11 (4 H, s), 6.87–6.35 (3 H, m), 6.22 (1 H, d, J = 15.4 Hz), 3.88 (1 H, m, D_2O exchange), 3.35 (3 H, s), 3.15–2.55 (4 H, m); MS m/e (FD) 464 (MH^+). Anal. ($C_{25}H_{24}N_2O_5S$), C, H, N.

***N*-Cyclohexyl-4-[*N*-methyl-*N*-[(*E*)-3-(3,4-dihydroxyphenyl)-2-propenoyl]amino]benzenesulfonamide (14)**: 89%; mp 224–225 °C (ethyl acetate–isopropyl ether); NMR (400 MHz, DMSO-d_6) δ 9.16 (2 H, br s), 7.88 (2 H, d, J = 8.6 Hz), 7.64 (1 H, br d, J = 7.0 Hz), 7.52 (2 H, d, J = 8.6 Hz), 7.38 (1 H, d, J = 15.9 Hz), 6.77 (1 H, br s), 6.69 (2 H, s), 6.13 (1 H, d, J = 15.9 Hz), 3.34 (3 H, s), 3.00 (1 H, m), 1.80–1.00 (10 H, m); MS m/e (FD) 430 (MH^+). Anal. ($C_{22}H_{26}N_2O_5S$), C, H, N.

Method B. *N*-(1-Benzyl-4-piperidinyl)-4-[*N*-methyl-*N*-[(*E*)-3-[4-(methylsulfonyl)phenyl]-2-propenoyl]amino]-benzenesulfonamide (3). To a suspension of 3-(4-methylsulfonyl)cinnamic acid[12d] (1.56 g, 6.67 mmol) and dimethylformamide (2 drops) in dichloromethane (20 mL) was added dropwise oxalyl chloride (2.01 mL, 23.3 mmol) at room temperature, and the mixture was stirred for 1 h. The resulting solution was concentrated to afford the acid chloride as a white solid. A solution of this acid chloride in dichloromethane (25 mL) was slowly added to a solution of anilide **7t** (2.62 g, 6.06 mmol) in pyridine (25 mL) at 0 °C. The mixture was stirred at room temperature for 2 h, poured into water, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography

(eluted with chloroform:methanol:aqueous ammonia = 98:2:0.2) and recrystallization from ethyl acetate–ether to give amide **3** as white crystals (2.60 g, 76%): mp 189–190 °C; NMR (400 MHz, $CDCl_3$) δ 7.95 (2 H, d, J = 8.6 Hz), 7.88 (2 H, d, J = 8.4 Hz), 7.74 (1 H, d, J = 15.4 Hz), 7.50 (2 H, d, J = 8.4 Hz), 7.36 (2 H, d, J = 8.6 Hz), 7.27 (5 H, m), 6.45 (1 H, d, J = 15.4 Hz), 4.27 (1 H, d, J = 7.7 Hz), 3.46 (2 H, s), 3.46 (3 H, s), 3.27 (1 H, m), 3.02 (3 H, s), 2.75 (2 H, br d, J = 12.1 Hz), 2.05 (2 H, t like, J = 8.0 Hz), 1.80 (2 H, m), 1.52 (2 H, m); MS m/e (FAB) 568 (MH^+), 172 (base). Anal. ($C_{29}H_{33}N_3O_5S_2$) C, H, N.

***N*-[1-(2-Phenylethyl)-4-piperidinyl]-4-[*N*-methyl-*N*-[(*E*)-3-[4-(methylsulfonyl)phenyl]-2-propenoyl]amino]benzenesulfonamide (4)**: 74%; mp 173–176 °C (ether–ethyl acetate); NMR (400 MHz, $CDCl_3$) δ 7.96 (2 H, d, J = 8.6 Hz), 7.87 (2 H, d, J = 8.4 Hz), 7.74 (1 H, d, J = 15.6 Hz), 7.50 (2 H, d, J = 8.4 Hz), 7.37 (2 H, d, J = 8.6 Hz), 7.28 (2 H, m), 7.17 (3 H, m), 6.46 (1 H, d, J = 15.6 Hz), 4.68 (1 H, d, J = 6.0 Hz), 3.46 (3 H, s), 3.28 (1 H, m), 3.01 (3 H, s), 2.86 (2 H, br d, J = 12.3 Hz), 2.75 (2 H, m), 2.56 (2 H, m), 2.11 (2 H, br t, J = 8.0 Hz), 1.87 (2 H, m), 1.55 (2 H, m); MS m/e (FAB) 582 (MH^+), 154 (base). Anal. ($C_{30}H_{35}N_3O_5S_2$) C, H, N.

***N*-[2-(6-Methyl-2-pyridyl)ethyl]-4-[*N*-methyl-*N*-[(*E*)-3-[4-(methylsulfonyl)phenyl]-2-propenoyl]amino]benzenesulfonamide hydrochloride (28)**: 79%; mp 169–175 °C (ethyl acetate); NMR (400 MHz, DMSO-d_6) δ 7.94 (2 H, d, J = 8.6 Hz), 7.83 (2 H, d, J = 8.2 Hz), 7.72 (1 H, d, J = 15.6 Hz), 7.48 (3 H, m), 7.34 (2 H, d, J = 8.6 Hz), 7.00 (1 H, d, J = 7.5 Hz), 6.90 (1 H, d, J = 7.7 Hz), 6.60 (1 H, br t), 6.43 (1 H, d, J = 15.6 Hz), 3.44 (3 H, s), 3.42 (2 H, m), 3.02 (3 H, s), 2.94 (2 H, t, J = 6.0 Hz), 2.48 (3 H, s); MS m/e (FAB) 514 (MH^+, base). Anal. ($C_{25}H_{27}N_3O_5S_2 \cdot HCl \cdot 0.2H_2O$) C, H, N.

Biology. 1. Rabbit Heart Membrane Fraction Preparation. Male NZW strain rabbits obtained from Clean Experimental Animals Center (Saitama, Japan) were anesthetized with sodium pentobarbital (40–50 mg/kg) given intravenously. The hearts were excised and washed several times with cold physiological saline. The ventricles, which were isolated from the hearts, were weighed, cut by scissors, and homogenized with 5 volumes (mL/g tissue) of cold homogenate buffer (0.25 M sucrose, 20 mM Tris-HCl, pH 8.0) using a Hiscotron homogenizer (Nihonseimitsu-kogyo) for 30 s at a quarter of maximal speed. The homogenate was centrifuged at 1000g for 10 min. The pellet was discarded. The supernatant was decanted through cheese cloth and centrifuged at 105 000g for 1 h. The homogenation and the centrifugation mentioned above were carried out at 0–4 °C. The pellet was suspended in cold homogenate buffer. The obtained suspension was adjusted to 5 mg of protein/mL and used as the membrane fraction for the PLA_2 activity assay. Protein was determined by the method of Lowry et al.,[17] using bovine serum albumin as a standard.

2. Assay of PLA_2 Activity. One hundred millimoles of $CaCl_2$ or EGTA was added to one-hundredth the volume of the membrane fraction. PLA_2 activity was assayed by using 0.2 mL of the membrane fraction in the presence or absence of various test samples. Two microliters of test sample was added to each tube as DMSO solutions. To the control tubes, 2 μL of DMSO was added. Reactions were initiated by the addition of 5 μL of radiolabeled phosphatidylcholine (1-palmitoyl-2-([1-^{14}C]arachidonyl)phosphatidylcholine; 8 mM ethanol solution; 1180 MBq/mL) as a substrate and carried out at 37 °C for 30 min. Reaction was stopped by the addition of 1 mL of Dole reagent.[18] Four-hundred microliters of distilled water and 600 μL of heptane were then added and the solution was shaken for 10 s. After centrifugation, at 20 °C for 7 min at 3000 rpm, 450 μL of the upper phase was pipetted into another tube containing 1.5 mL of heptane and 95–110 mg of silicic acid powder, and then the solution was shaken for 30 s. After centrifugation at 20 °C for 3 min at 3000 rpm, 1 mL of the heptane phase was transferred into a scintillation vial. Ten milliliters of scintillation fluid (ACS II; Amersham, Canada) was added to each vial. Radioactivity of the extracted arachidonic acid was counted in an Aloka Model LSC-700 or a LSC-3500 scintillation counter with an efficacy of about 90%. The mean value of the triplicated measurements was used as PLA_2 activity.

3. Effects on the Protection of Ischemic Myocardial Injury in Coronary-Ligated Rats. The myoglobin-depleted area of the rat ventricular myocardium in the early phase of ischemia was measured as an index of myocardial infarction with the use of an immunohistochemical technique and an image analyzer. Sprague-Dawley rats (180–260 g) were anesthetized with diethyl ether, and the chest was opened in the left fifth intercostal space and the left main coronary artery was ligated at 1 to 2 mm from its origin according to a modification of the method reported by Selye et al.[19] The heart was then replaced in the thoracic cavity and the thoracic wall was closed by a purse-string suture. The thoracic cavity was open for 40 to 50 s during which period no artificial respiration was made. Of the animals subjected to coronary occlusion, the rats that died within the first 3 h after the ligation were omitted from the experimental protocol. The duration from coronary ligation to sacrifice was 3 h. Tested samples were administrated intravenously as an aqueous solution or an acidic aqueous solution 15 min prior to the ligation, whereas the vehicles were administrated intravenously in the control rats. Staining and morphometric analysis were carried out according to the method reported previously.[14]

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(17) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.

(18) Dole, V. P.; Meinertz, H. *J. Biol. Chem.* **1960**, *235*, 2595.

(19) Selye, H.; Bajusz, E.; Grasso, S.; Medell, P. *Angiology* **1960**, *11*, 398.