Regioselective Cleavage Reaction of the Aromatic Methylenedioxy Ring. VI.[1] Synthesis of Phenothiazine Analogues by Using the Cleavage Reaction with Sodium Methoxide–Thiols in Dimethyl Sulfoxide and Evaluation of Their Biological Activities

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The reactions of aromatic methylenedioxy compounds containing electron-withdrawing groups with sodium methoxide–thiols in dimethyl sulfoxide gave 3- and 4-hydroxybenzene derivatives in good yield by regioselective attack of the thiolate ions on the methylenedioxy ring. The formation mechanism and the reactivity of thiolate ions in the cleavage reaction of the methylenedioxy ring are discussed. Various biologically active compounds, 32a, 32d, 36b, 38b, 41b and 44—47, were prepared from the 4-hydroxybenzene derivatives and their Ca^{2+} antagonistic activities were evaluated. Among these compounds, 2-(2-bromophenylthiomethoxy)-10-(2-diethylaminoacetyl)-3-methoxyphenothiazine (46) showed the most potent Ca^{2+} antagonistic activity. Biological activity could be conveniently evaluated by measurement of the peak height of the vanadyl ion (+4 oxidation ion) signal produced by redox reaction between the phenothiazine derivatives and vanadate ion +5 oxidation ion) with ESR spectroscopy.

Keywords regioselective cleavage reaction; thiolate ion; calcium ion antagonistic activity; aromatic methylenedioxy ring; phenothiazine analogue; ESR spectroscopy

We have found[1,2] that regioselective 3,4-methylenedioxy ring cleavage reactions in aromatic formyl (CHO), nitro (NO_2) and acetyl (MeCO) compounds occur with the nucleophiles ($R'O^-$) of hard bases formed from the protic solvents (R'OH) in sodium alkoxides (RONa)–alcohols (R'OH) [R = R' or R ≠ R', R or R' = methyl (Me), isopropyl (Me_2CH), benzyl ($PhCH_2$), phenyl (Ph), and 4-methoxyphenyl (4-$MeOC_6H_4$)]-dipolar aprotic solvents [dimethyl sulfoxide (DMSO), dimethyl formamide (DMF) and hexamethylphosphoramide (HMPA)] systems, as shown in Chart 1. In this paper, we describe the regioselective cleavage reactions of aromatic methylenedioxy ring containing electron-withdrawing groups with the nucleophiles (thiolate ions) of soft bases formed in sodium methoxide (MeONa)–thiols (ethanethiol and **2a**—**2d**)–DMSO systems. The Ca^{2+} antagonistic activity of the synthesized compounds **31d**, **32a**, **32d**, **36b**, **38b**, **41b**, **44**—**47** and **50** was evaluated based on the inhibitory effect on the KCl-induced contraction of rat aorta. Further, the biological activity could be evaluated by measurement of the peak height due to the vanadyl ion formed in the redox reaction between vanadate ion and four phenothiazine drugs **26**—**29** or the synthesized compounds **32d**, **45**, **46**, **49** and **50** by electron spin resonance (ESR) spectroscopy.

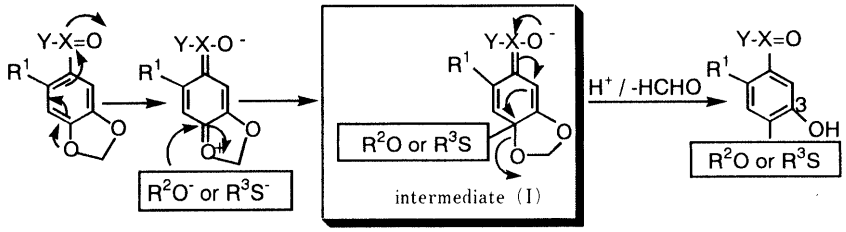
Cleavage of Aromatic Methylenedioxy Compounds (1, 4, 5, 8 and 9) with MeONa and Ethanethiol (EtSH) in DMSO The reaction of 6-bromopiperonal (**1**) with MeONa–EtSH in DMSO at 40 °C for 20 min gave a cleavage product **2** in 44.5% yield. Compound **2** showed infrared (IR) absorptions at 3400 (OH), 2890 and 1680 (CHO) cm^{-1}, and the proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed signals arising from a formyl group [δ 10.24 (s)], an ethyl group [δ 2.89 (2H, q, J = 7.3 Hz, $SC\underline{H}_2CH_3$) and δ 1.31 (3H, t, J = 7.3 Hz, $SCH_2C\underline{H}_3$)], H-5 [δ 7.63 (1H, s)], H-2 [δ 7.49 (1H, s), and a hydroxy group [δ 6.72 (lH, brs, OH, D_2O-exchangeable)]. Irradiation at δ 2.89 gave a 4.4% intramolecular nuclear Overhauser effect (NOE) increment in the signal at δ 7.63, which was assigned to H-5 by comparison with the ^{1}H-NMR spectral data [δ 1.23 (3H, t, J = 7.3 Hz, $SCH_2C\underline{H}_3$), 2.73 (2H, q, J = 7.3 Hz, $SC\underline{H}_2CH_3$), 4.68 (2H, s, $C\underline{H}_2OH$), 6.72 (lH, brs, OH, D_2O-exchangeable), 7.16 (1H, s, H-2), and 7.61 (1H, s, H-5)] of 2-bromo-4-ethylthio-5-hydroxybenzyl alcohol (**3**) prepared by the $NaBH_4$ reduction of **2**. In the ^{1}H-NMR spectrum of **3**, irradiations at δ 2.73 and 4.63 gave 3.5 and 3.3% NOE increments in the signals at δ 7.61 and 7.16, respectively. From the above findings, compound **2** was determined to be 2-bromo-4-ethylthio-5-hydroxybenzaldehyde. The reactions of 1,2-methylenedioxy-4-nitrobenzene (**4**) and 4,5-methylenedioxy-2-nitrobenzyl alcohol (**5**) with MeONa–EtSH in DMSO gave the 3-hydroxybenzene derivatives (**6** and **7**, respectively), as shown in Table I. The structures of **6** and **7** were deduced to be 4-ethylthio-3-hydroxynitrobenzene and 4-ethylthio-3-hydroxy-6-hydroxymethylnitrobenzene, respectively, from their physical and spectral data (Tables III and IV). On the other hand, the reactions of 6-nitropiperonal (**8**) and 1-bromo-4,5-methylenedioxy-2-nitrobenzene (**9**) with MeONa–EtSH in DMSO afforded only non-cleavage products, 6-ethylthiopiperonal (**10**) and 1-ethylthio-4,5-methylenedioxy-2-nitrobenzene (**11**), respectively, as

type I. synthesis of 3-hydroxybenzene derivatives

nucleophilic reagents : RONa-R^2OH or MeONa-R^3SH

(R=Me, $PhCH_2$; R^1=H, Br, I, Ph; R^2=Me, $PhCH_2$, Me_2CH, Me_3CCH_2; R^3=Et)

X = C, Y = H X = N, Y = O

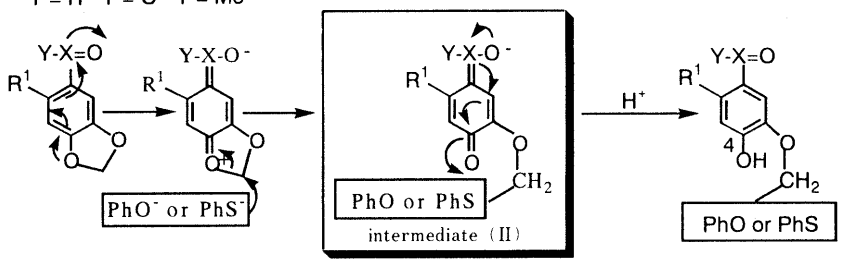


type II. synthesis of 3-hydroxybenzene derivatives

nucleophilic reagents : RONa-PhOH or MeONa-PhSH

(R=Me, $PhCH_2$, Ph; R^1=H, Br, I, Ph)

X = C, Y = H X = N, Y = O X = C, Y = Me



type III. synthesis of 3- and 4-hydroxybenzene derivatives

nucleophilic reagents : RONa-R^2OH

(R=R^2=Me, $PhCH_2$)

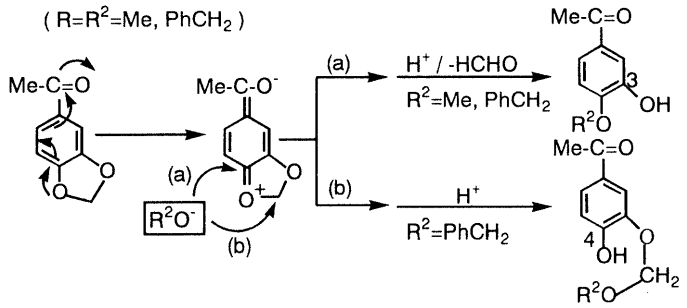
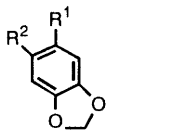
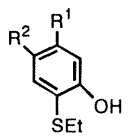


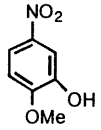
Chart 1



	R^1	R^2
1	CHO	Br
4	NO_2	H
5	NO_2	CH_2OH
8	CHO	NO_2
9	NO_2	Br
10	CHO	SEt
11	NO_2	SEt
12	CHO	H
15	CHO	SPh
16	CH_2OH	Br
18	NO_2	SPh



	R^1	R^2
2	CHO	Br
3	CH_2OH	Br
6	NO_2	H
7	NO_2	CH_2OH



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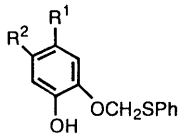


	R^1	R^2
13	CHO	Br
14	CHO	SPh
17	NO_2	SPh
19	NO_2	H
20	NO_2	CH_2OH
21	CHO	H

Chart 2

TABLE I. Cleavage of **1**, **4**, **5**, **8**, and **9** with MeONa and EtSH in DMSO[a]

Compound (mmol)		MeONa (mmol)	EtSH (ml)	DMSO (ml)	Product	Yield (%)[b]
1	0.43	0.90	0.45	0.5	**2**	44.5
4	0.45	0.90	0.45	0.5	**6**	87.6
5	0.45	0.90	0.45	0.5	**7**	55.7
8	0.45	0.90	0.45	0.5	**10**[c]	63.4
9	0.45	0.90	0.45	0.5	**11**[c]	78.5

a) Stirred at 40 °C for 20 min. *b*) Based on the initial amount of starting material. *c*) Non-cleavage compound.

shown in Tables I, III and IV.

Cleavage of Aromatic Methylenedioxy Compounds (1, 4, 5, 8, 9 and 12) with MeONa and PhSH in DMSO The reaction of **1** with MeONa–PhSH in DMSO at 130 °C for 3 min gave two phenolic products **13** and **14**, together with two non-cleavage products **15** and **16**, as shown in Table II. The reaction of **8** under the same conditions gave a cleavage product **14** and a non-cleavage product **15**, as shown in Table II. Compound **13** showed IR absorptions at 3350 (OH), 2850 and 1660 (CHO) cm^{-1}. The ^{1}H-NMR spectrum showed the presence of a formyl group at δ 10.16 (s), H-2 at δ 7.49 (s), H-5 at δ 7.21 (s), and a phenylthiomethoxy group at δ 7.38 (5H, m, aromatic H) and δ 5.54 (2H, s, $OC\underline{H}_2S$). Irradiation of the proton at δ 5.54 gave a 2.0% NOE increment in the signal at δ 7.49. From the above findings, the structure of **13** was determined to be 2-bromo-4-hydroxy-5-phenyl-thiomethoxybenzaldehyde. Compound **14** showed IR absorptions at 3350 (OH), 2840 and 1660 (CHO) cm^{-1}, and the ^{1}H-NMR spectrum showed the presence of a formyl group at δ 10.33 (s), H-2 at δ 7.50 (s), H-5 at δ 6.83 (s) and a phenylthiomethoxy group at δ 7.43 (10H, m, aromatic H, overlapping with another aromatic H) and δ 5.55 (2H, s, $OC\underline{H}_2SPh$). A 12.8% NOE increment in the signal at δ 7.50 was observed on irradiation at δ 5.55. Consequently, the structure of **14** was established to be 4-hydroxy-2-phenylthio-5-phenylthiomethoxybenzaldehyde. Similarly, the structure of **15** was established to be 6-phenylthiopiperonal from the physical and spectral data (Tables III and IV). The reaction of 1-bromo-4,5-methylenedioxy-2-nitrobenzene (**9**) with MeONa–PhSH in DMSO gave 4-nitro-5-phenylthio-2-phenylthiomethoxyphenol (**17**) and a non-cleavage compound **18**, as shown in Table II. The reactions of 1,2-methylenedioxy-4-nitrobenzene (**4**), 4,5-methylenedioxy-2-nitrobenzyl alcohol (**5**), and piperonal (**12**) with MeONa–PhSH in DMSO gave the 4-hydroxybenzene derivatives (**19**—**21**, respectively), as shown in Table II. The structures of compounds **19**—**21** were established to be 4-hydroxy-3-phenylthiomethoxynitrobenzene, 4-hydroxy-6-hydroxymethyl-3-phenylthiomethoxynitrobenzene, and 4-hydroxy-3-phenylthiomethoxybenzaldehyde, respectively, from their physical and spectral data (Tables III—V).

Mechanism and Reactivity of the Regioselective Cleavage Reaction of Aromatic Methylenedioxy Ring with MeONa–EtSH or MeONa–PhSH in DMSO We have previously reported[2d] that the order of reactivity of the protic solvents used in the cleavage reactions of the methylenedioxy ring is PhOH > 4-$MeOC_6H_4OH$ > MeOH > $PhCH_2OH$, *i.e.*, the inverse of the order of pK_a values of the protic solvents. To confirm the reactivity of ethanol (EtOH, pK_a 16) and EtSH (pK_a 10.6) or PhOH (pK_a 10.0) and PhSH (pK_a 6.4) towards the aromatic methylenedioxy ring in the MeONa–DMSO system, we examined the following cleavage reactions. The cleavage reaction of **4** with MeONa–EtSH–EtOH in DMSO at 40 °C for 20 min gave only **6** in 90% yield, and the cleavage reaction of **4** with MeONa–PhSH–PhOH in DMSO at 130 °C for 20 min gave only **19** in 74.9% yield. These results suggest that the reactivity of nucleophiles in the cleavage reactions of the methylenedioxy ring may be controlled by the relative polarizability, as seen in usual aromatic nucleophilic substitution reactions.[3]

TABLE II. Cleavage of **1**, **4**, **5**, **8**, **9**, and **12** with MeONa and PhSH in DMSO

Compound (mmol)		MeONa (mmol)	PhSH (ml)	DMSO (ml)	Product	Yield (%)[a]
1[b]	0.43	0.90	0.45	0.5	**13**	40.1
					14	20.7
					15[c]	25.7
					16[c]	4.1
1[b]	0.43	1.36	0.45	0.5	**13**	33.6
					14	21.7
					15[c]	21.8
					16[c]	4.3
4[b]	0.45	0.90	0.45	0.5	**19**	69.0
4[b]	0.45	1.36	0.45	0.5	**19**	73.8
5[d]	0.45	1.36	0.45	0.5	**20**	49.4
8[b]	0.45	1.36	0.45	0.5	**14**	65.9
					15[c]	16.4
9[d]	0.45	1.36	0.45	0.5	**17**	70.2
					18[c]	28.2
12[d]	0.45	0.90	0.45	0.5	**21**	49.7
12[d]	0.45	1.36	0.45	0.5	**21**	72.0

a) Based on the initial amount of starting material. *b*) Stirred at 130 °C for 3 min. *c*) Non-cleavage compound. *d*) Stirred at 130 °C for 10 min.

The formation mechanism of the thiolate ions (RS^-) produced in the cleavage reactions may be understood as follows. In general, it is well known[4] that thiol compounds (RSH) in dipolar aprotic solvents are readily converted into disulfide derivatives (RS–SR) and form thiolate ions (RS^-) with base. The disulfide derivatives are readily transformed into thiolate ion (RS^-) and thioether derivatives (RSNu) by nucleophilic reagents (soft base; $Nu^- = RS$, CN, SO_3, Ph_3P).[5] The cleavage reaction of the aromatic methylenedioxy ring by MeONa–PhSH in DMSO gave diphenyl disulfide (mp 57.0—59.5 °C, PhS–SPh) together with the cleavage products. On the other hand, the cleavage reaction of **4** with MeONa–PhSSPh in DMSO at 130 °C for 3 min gave 2-methoxy-5-nitrophenol (**22**) in 7.2% yield along with a cleavage product **19** in 49.5% yield, which indicated the formation of thiolate ion, *e.g.*, as in Eq. 1.

$$MeO^- + PhS\text{–}SPh \rightleftharpoons MeO\text{–}SPh + PhS^- \quad (1)$$

The formation of compound **22** can be explained as follows. The MeOSPh formed in Eq. 1 would be converted easily into MeO^- and disulfide by the thiolate ion, because MeO^- is a hard base and PhS^+ is a soft acid.

TABLE III. Physical and Spectroscopic Properties of **2**, **6**, **7**, **10**, **11**, **13**, **14**, **15**, and **17**—**21**

Compound	mp (°C) (Recrystn. solvent)	Formula	Analysis (%) Calcd (Found)			IR (KBr) cm^{-1}		
			C	H	N	OH	CHO	NO_2
2	119.5—122.5 (benzene)	$C_9H_9BrO_2S$	41.40 (41.64	3.47 3.38)		3400	2890 1680	
6	90.0—93.0 (benzene–*n*-hexane)	$C_8H_9NO_3S$[a]		199.0300 (199.0288)		3390		1580 1340
7	103.0—106.0 ($CHCl_3$)	$C_9H_{11}NO_4S$[a]		299.0406 (299.0403)		3350		1580 1330
10	59.0—62.0 (benzene–*n*-hexane)	$C_{10}H_{10}O_3S$	57.13 (57.06	4.79 4.78)		3200	2880 1680	
11	118.5—120.5 (benzene–$CHCl_3$)	$C_9H_9NO_4S$	47.57 (47.69	3.99 3.89	6.16 5.86)			1500 1300
13	123.0—124.5 ($CHCl_3$–*n*-hexane)	$C_{14}H_{11}BrO_3S$	49.56 (49.68	3.24 3.11)		3350	2850 1660	
14	111.5—114.0 ($CHCl_3$–*n*-hexane)	$C_{20}H_{16}O_3S_2$	65.19 (64.74	4.38 4.19)		3350	2840 1660	
15	60.0—61.0 (Et_2O–*n*-hexane)	$C_{14}H_{10}O_3S$	65.10 (65.06	3.90 3.63)			2850 1680	
17	137.5—141.0 (CCl_4–$CHCl_3$)	$C_{19}H_{15}NO_4S_2$[a]		385.0440 (385.0423)		3400		500 1320
18	136.0—138.0 (CCl_4–MeOH)	$C_{13}H_9NO_4S$	56.72 (56.54	3.30 3.15	5.09 4.78)			1500 1300
19	Amorphous	$C_{13}H_{11}NO_4S$[a]		277.0409 (277.0427)		3440		1520 1340
20	Oil	$C_{14}H_{13}NO_5S$[a]		307.0512 (307.0477)		3370[b]		1520[b] 1320[b]
21	85.0—88.0 (benzene–*n*-hexane)	$C_{14}H_{12}O_3S$	64.62 (64.75	4.62 4.53)		3100	2840 1660	

a) Established by high resolution mass spectrometry. *b*) Measured as a liquid film.

TABLE IV. NMR Data[a] of **2**, **6**, **7**, **10**, **11**, **13**, **14**, **15**, **17**—**21**, **30a**, **30b**, **32b**, and **32c** ($CDCl_3$, δ)

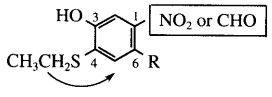
Compound	Aromatic H			CHO	SCH_2CH_3	SCH_2CH_3	OCH_2SPh	OCH_2O
	H-2	H-5	H-6					
2	7.49	7.63		10.24	2.89 (q, 7.3)	1.31 (t, 7.3)		
6	7.81 (d, 2.4)	7.57 (d, 8.5)	7.76 (dd, 8.5, 2.4)		2.86 (q, 8.0)	1.28 (t, 8.0)		
7[b]	7.55	7.54			3.03 (q, 8.0)	1.38 (t, 8.0)		
10	7.17	6.80		10.27	2.85 (q, 8.0)	1.60 (t, 8.0)		5.93
11	7.57	6.70			2.88 (q, 8.0)	1.37 (t, 8.0)		6.03
13	7.49	7.21		10.16			5.54	
14	7.50	6.83		10.33			5.55	
15	7.27	6.67		10.33			5.93	
17	7.88	6.32					5.53	
18	7.57	6.12						5.92
19[b]	7.81 (d, 2.4)	6.98 (d, 8.9)	7.91 (dd, 8.9, 2.4)				5.57	
20	7.77	[c]					5.55	
21	7.46 (d, 1.9)	7.06 (d, 8.6)	7.48 (dd, 8.6, 1.9)	9.80			5.56	
30a	7.78 (d, 2.2)	6.87 (d, 9.0)	7.83 (dd, 9.0, 2.2)				5.63	
30b	7.67 (d, 2.0)	6.87 (d, 8.0)	7.85 (dd, 8.0, 2.0)				5.23	
32b[b]	7.88	6.14					5.60	
32c	7.78	6.25					5.35	

a) Signals are singlets except where otherwise indicated in parentheses. The numerical values in parentheses are coupling constants in Hz. *b*) In $CDCl_3$ and CD_3OD. *c*) Obscured signal.

TABLE V. NOE Increments in the H-2 and H-5 Signals of **2**, **3**, **6**, **7**, **13**, **14**, **17**, and **19**—**21**[a]

NOE increment (%) of	Compounds									
	2	**3**	**6**	**7**	**13**	**14**	**17**	**19**	**20**	**21**
H-2		3.3 (4.68)			2.0 (5.54)	12.8 (5.55)	11.2 (5.53)	24.0 (5.57)	8.4 (5.50)	3.8 (5.56)
H-5	4.4 (2.89)	3.5 (2.73)	2.0 (2.86)	2.1 (4.91)	6.8 (3.03)					

a) Irradiation position (δ) in parenthesis.



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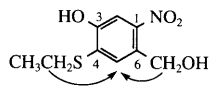

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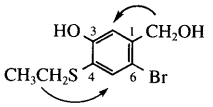

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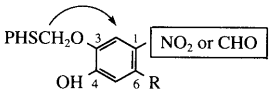

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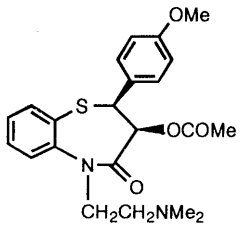

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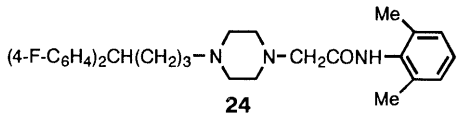

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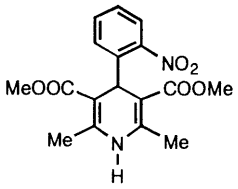

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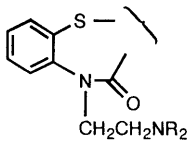
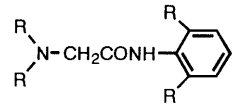
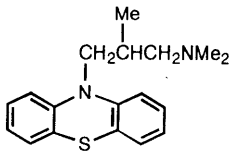


partial structure A



partial structure B



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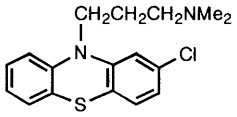

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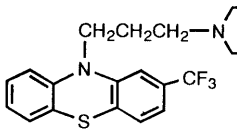

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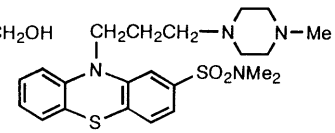


29

Chart 3

As the cleavage reactions of the aromatic methylenedioxy ring with MeONa–PhSH in DMSO gave only thioether compounds as the cleavage products, the formation mechanism of the thiolate ion produced in the cleavage reactions is considered to be described by Eqs. 2—5.

$$MeO^- + RSH \rightarrow MeOH + RS^- \quad (2)$$

$$2PhSH + DMSO \rightarrow Me_2S + H_2O + PhS\text{–}SPh \quad (3)$$

$$2PhS^- + O_2 \rightarrow PhS\text{–}SPh + O_2^{2-} \quad (4)$$

$$\underline{PhS}^- + PhS\text{–}SPh \rightarrow \underline{PhS}\text{–}SPh + PhS^- \quad (5)$$

Synthesis of Some Biologically Active Compounds Calcium antagonistic drugs such as diltiazem (**23**), lidoflazine (**24**) and nifedipine (**25**), and neuroleptic drugs such as phenothiazine compounds (**26**—**29**) contain characteristic partial structures A and B (Chart 3) that can be readily prepared by using our cleavage reactions. Hence, by the method shown in Charts 4—6, we synthesized compounds **31d**, **32a**, **32d**, **36b**, **38b** and **41b** of group I and compounds **44**—**47**, **49** and **50** of group II which contain the partial structures A and B.

As shown in Table VI, the regioselective cleavage reactions of **4** with MeONa–thiols (**2b** and **2d**) in DMSO gave 4-hydroxybenzene derivatives (**30a** and **30b**, respectively) in good yield, and the regioselective cleavage reactions of **9** with MeONa–thiols (**2b**, **2c** and **2d**) in DMSO gave the 4-hydroxybenzene derivatives (**32a**, **32b** and **32c**, respectively) in good yield, together with the non-cleavage compounds **31a**, **31b** and **31c**. Acylation of

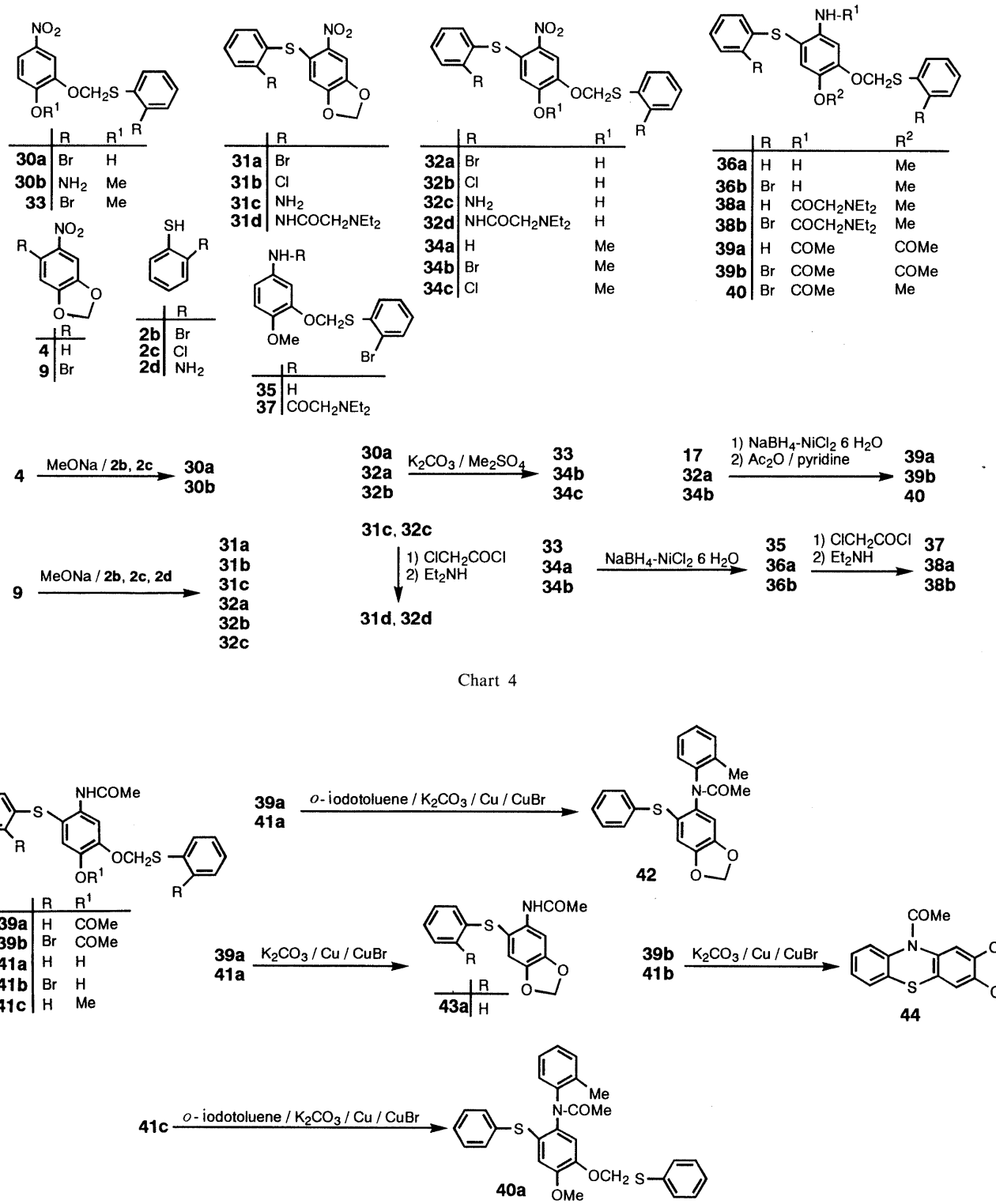


Chart 4

Chart 5

31c and **32c** with chloroacetyl chloride ($ClCOCH_2Cl$), followed by condensation with diethylamine (Et_2NH) gave **31d** and **32d**, respectively. The structures of **30a**, **30b**, **31d**, **32a**, **32b**, **32c** and **32d** were established on the basis of their physical and spectral data (Tables IV, VII and VIII). Compounds **30a**, **32a** and **32b** were methylated with K_2CO_3–Me_2SO_4 in dry acetone to give **33**, **34b** and **34c**, respectively. The reduction of compounds **17**, **32a** and **34b** with $NaBH_4$–$NiCl_2 \cdot 6H_2O$,[6] followed by acylation with Ac_2O–pyridine gave **39a**, **39b** and **40**, respectively. The reduction of compounds **33**, **34a** and **34b** with $NaBH_4$–$NiCl_2 \cdot 6H_2O$ gave amino derivatives (**35**, **36a** and **36b**, respectively). Acylation of **35**, **36a** and **36b** with $ClCOCH_2Cl$, followed by condensation with Et_2NH gave **37**, **38a** and **38b**, respectively. We found that compounds **39a**, **39b**, **41a** and **41b** are easily converted to aromatic

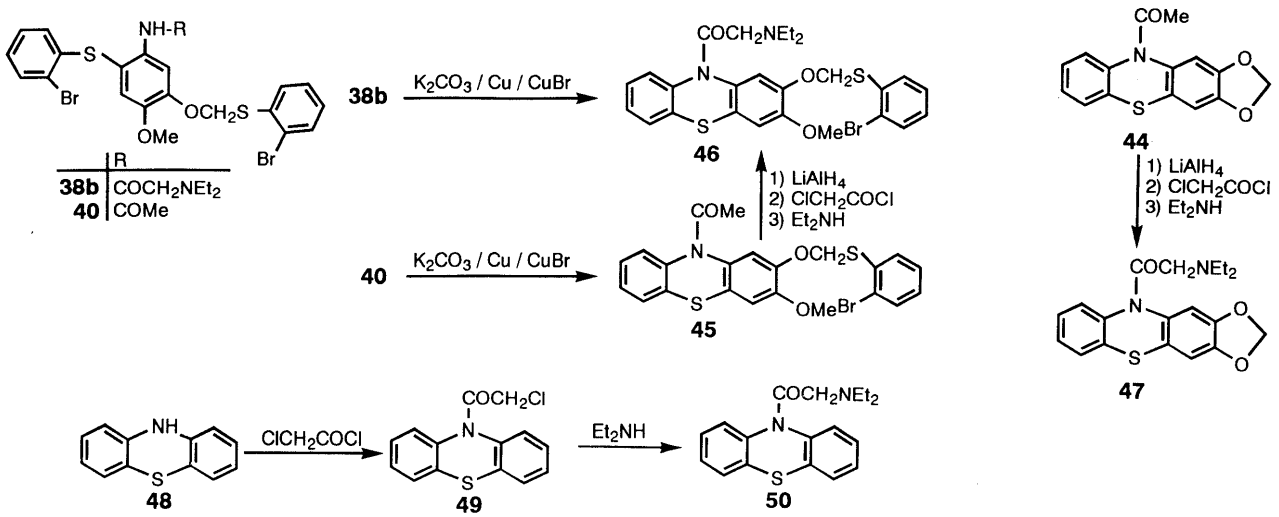


Chart 6

TABLE VI. Cleavage of **4** and **9** with MeONa and Thiol Compounds (**2b**, **2c**, and **2d**) in DMSO

Compound (mmol)		MeONa (mmol)	Thiol compound (ml)		DMSO (ml)	Product	Yield (%)[a]
4[b]	1.80	5.0	**2b**	(2.0)	2.5	**30a**	75.8
4[b]	1.97	3.1	**2d**	(1.7)	2.0	**30b**	72.7
9[d]	0.90	2.0	**2b**	(1.75)	1.0	**32a**	80.7
						31a[c]	11.0
9[d]	0.45	1.36	**2c**	(1.75)	0.5	**32b**	71.4
						31b[c]	17.6
9[d]	1.0	2.7	**2d**	(1.7)	1.5	**32c**	48.6
						31c[c]	18.4

a) Stirred at 140 °C for 1 h. *b*) Based on the initial amount of starting material. *c*) Non-cleavage compound. *d*) Stirred at 130 °C for 30 min.

methylenedioxy compounds **43a**, **43b** and **44** by using K_2CO_3–Cu powder–CuBr in nitrobenzene (Ullmann reaction),[7] and that the reaction of compounds **39a** or **41a** with *o*-iodotoluene–K_2CO_3–Cu powder–CuBr in nitrobenzene gave **42** as shown in Chart 5. On the other hand, the reaction of **40** under the same conditions gave **40a**. Therefore, the synthesis of a new phenothiazine compound **46** was achieved together with the syntheses of compounds **47**, **49** and **50**, by the method shown in Chart 6. The structures of these compounds were established on the basis of the physical and spectral data (Tables VII, VIII and Experimental).

Biological Results and Discussion

Membrane Ca^{2+} channels mediating Ca^{2+} entry are classified into two major types,[8] receptor-operated Ca^{2+} channels (ROCs) and potential-dependent Ca^{2+} channels (PDCs). ROCs are associated with membrane receptors and are activated by specific agonist-receptor interactions. PDCs are activated by membrane depolarization, and KCl-induced contractions of rat aorta are entirely dependent on Ca^{2+} influx through PDCs in the membrane. Ca^{2+} channel antagonists such as **23**, **24** and nifedipine (**25**) exert their actions mainly at the PDCs. Hence, the order 50% inhibitory concentrations (IC_{50}) for inhibition of KCl-induced contraction of isolated rat aorta strips were determined for group A (**31d**, **32a**, **32d**, **36b**, **38b** and **41b**) and group B (**44**—**47**, and **50**) together with **23** and chloropromazine (CPZ, **27**), as shown in Table IX. The order of the IC_{50} value was **41b** > **32d** > **31d** > **32a** ≫ **36b**, **38b** for compounds of group A and was **46** > **27** > **45** > **47** > **50** > **44** for compounds of group B. Nifedipine (**25**) inhibits noradrenaline contraction of rat aorta, but does not alter that of rabbit aorta.[9] The IC_{50} value (6.2×10^{-7} M) for inhibition of KCl-induced contractions of rat aorta for the most potent compound (**46**) among the synthesized compounds was lower than that (3×10^{-6} M) of **27**. Phenothiazine drugs have α-adrenoceptor-blocking and anti-histaminic actions. In fact, noradrenaline-induced contractions of rabbit aorta and histamine-induced contractions of isolated guinea-pig ileum were suppressed by **27** at significantly lower concentrations of 10^{-7}—10^{-6} and 3×10^{-9}—3×10^{-7} M, respectively, than the IC_{50} value (3×10^{-6} M)[10] for inhibition of KCl-induced contractions of rat aorta. On the other hand, the new phenothiazine compound **46** inhibited KCl-induced contraction of rat aorta at the low concentration of approximately 6.2×10^{-7} M and showed almost no inhibitory effect on the noradrenaline-induced contraction of rabbit aorta at 10^{-7}—10^{-6} M or on the histamine-induced contraction of guinea-pig ileum at 10^{-6}—10^{-5} M. Further, compound **46** showed no effect on heart contraction (HC) and heart rate (HR) in isolated guinea pig atria at the concentration of 10^{-7}—10^{-5} M.

TABLE VII. Physical and Spectroscopic Properties of **30a**, **30b**, **31d**, **32a**, **32b**, **32c**, **32d**, **38b**, **41b**, **44**—**47**, and **50**

Compound	mp (°C)	Formula	Analysis (%) Calcd (Found)			IR (KBr) cm^{-1}			
			C	H	N	OH	NHCO	NO_2	NH_2
30a	109.0—112.0 (benzene–$CHCl_3$)	$C_{13}H_{10}BrNO_4S$	43.84 (44.13	2.81 2.89	2.93 3.21)	3400		1500 1340	
30b	175.0—176.0 (benzene)	$C_{13}H_{12}N_2O_4S$	53.43 (53.22	4.10 4.05	9.58 9.46)	3480		1515 1310	3480 3320
31d	138.0—141.0 (benzene–$CHCl_3$)	$C_{19}H_{21}N_3O_5S$[a]		403.2032 (403.2029)			3200 1680	1520 1330	
32a	156.0—159.0 (benzene)	$C_{19}H_{13}Br_2NO_4S_2$[a]		540.8654 (540.8687) 544.8611 (544.8610)		3370		1510 1310	
32b	139.0—142.0 (benzene)	$C_{19}H_{13}Cl_2NO_4S_2$[a]		438.9632 (438.9650) 442.9573 (442.9572)		3370		1510 1310	
32c	154.0—156.0 (benzene)	$C_{19}H_{17}N_3O_4S_2$	54.92 (54.87	4.12 4.31	10.11 10.00)	3250		1517 1332	3475 3432 3383 3346
32d	192.0—196.0 (acetone)	$C_{31}H_{39}N_5O_6S_2$	58.01 (57.93	6.13 6.29	10.91 10.63)	3450	3208 1680	1520 1310	
38b	141.5—156.0 (benzene–*n*-hexane)	$C_{26}H_{28}Br_2N_2O_3S_2$	48.76 (48.42	4.41 4.19	4.37 4.10)		3200 1660		
41b	124.0—126.0 (benzene)	$C_{21}H_{17}Br_2NO_3S_2$[a]		552.9018 (552.9043) 554.9017 (554.9052)		3350	3350 1660		
44	Amorphous	$C_{15}H_{11}NO_3S$[a]		285.0457 (285.0444)			1680		
45	128.5—131.0 (diethyl ether)	$C_{22}H_{18}BrNO_3S_2$[a]		486.9982 (486.9910) 488.9893 (488.9914)			1680		
46	Oil	$C_{26}H_{27}BrN_2O_3S_2$[a]		558.0647 (558.0719) 560.0628 (560.0635)			1675[b]		
47	Amorphous	$C_{19}H_{20}N_2O_3S$[a]		356.1196 (356.1201)			1680		
50	Amorphous	$C_{18}H_{20}N_2OS$[a]		312.1298 (312.1290)			1680		

a) Established by high-resolution mass spectrometry. *b*) Measured as a liquid film.

TABLE VIII. NMR Data[a] for **31d**, **32a**, **32d**, **36b**, **38b**, and **41b** ($CDCl_3$, δ)

Compound	H-2	H-5	H-6	OCH_2SPh	OCH_2O	$COCH_2N$	NCH_2CH_3	NCH_2CH_3	Others
31d	7.73	6.07			6.01	2.99	2.36 (q, 7.1)	0.80 (t, 7.1)	
32a[b]	7.88	6.02		5.68					
32d	7.88	5.42		5.31		2.98, 3.17	2.38, 2.62 (each q, 8.0)	0.79 (each t, 8.0)	
36b	6.95	6.47		5.57					3.70 (OMe)
38b	8.50	7.07		5.63		2.98	2.71 (q, 8.0)	0.80 (t, 8.0)	3.76 (OMe)
41b	8.23	[c]		5.60					2.07 (NHCOMe)

a) Signals are singlets except where otherwise indicated in parentheses. The numerical values in parentheses are coupling constants in Hz. *b*) In $CDCl_3$ and CD_3OD. *c*) Obscured signal.

These results suggest that the action of compound **46** on rat aorta may be based on inhibition of Ca^{2+} influx through PDCs. Thus, compound **46** is an interesting new phenothiazine compound, which shows Ca^{2+} antagonistic effect without any other non-specific inhibition. Syntheses of other new compounds are in progress, with the aim of

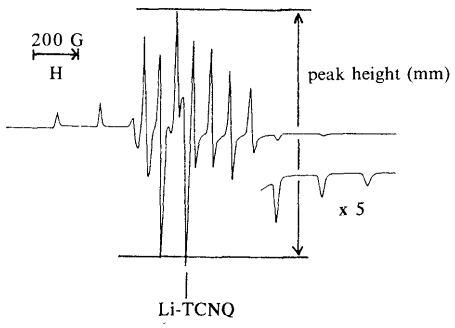
TABLE IX. IC_{50} Values of **31d**, **32a**, **32d**, **36b**, **38b**, **41b**, **44**—**47**, **50**, Diltiazem (**23**), and Chloropromazine (**31**) against the Contraction of Isolated Rat Aorta Strips Induced by 40 mM KCl

Compound	IC_{50} (M)[a]	Potency ratio[b]
31d	3.6×10^{-6}	1/19
32a	$>10^{-5}$	>1/53
32d	3.3×10^{-6}	1/17
36b	$\gg10^{-5}$	≫1/53
38b	$\gg10^{-5}$	≫1/53
41b	3.2×10^{-6}	1/17
44	$\gg10^{-5}$	≫1/53
45	6.2×10^{-6}	1/33
46	6.2×10^{-7}	1/3
47	7.2×10^{-6}	1/38
50	$>10^{-5}$	>1/53
23	1.9×10^{-7}	1
27	1.4×10^{-6}	1/7

a) IC_{50} values were calculated by regression analysis of the dose–response curves. *b*) IC_{50} values of test compounds were divided by that of diltiazem (**23**).

TABLE X. Peak Height of Vanadyl Ion at 77 K (Amplitude 2000) of **25**—**29**, **32d**, **45**, **46**, **49**, and **50**

Compound	Peak height (mm)
25	240
26	8000
27	6080
28	4560
29	4160
32d	75
45	1520
46	375
49	4000
50	435



Conditions: 1) 1 mM compound (0.25 ml), 1 mM $NaVO_3$ (0.25 ml), 0.1 M sodium phosphate buffer (pH 7.5, 2.0 ml). 2) 4 M HCl (0.3 ml); reaction time, 30 min.

finding superior selective Ca^{2+} antagonists.

As phenothiazine drugs show various activities, we have to perform various pharmacological experiments to deduce the biological activity of the drugs. If the biological activity of phenothiazine drugs could be deduced by spectrometry, identification of compounds of interest would be greatly simplified. Therefore, we carried out the following experiments. It has been suggested in a number of studies[11] that the phenothiazine radical cations are involved in the biological effects of the phenothiazine drugs. Cantley *et al.*[12] reported that the vanadate ion (+5 oxidation state) is a potent inhibitor of Na^+, K^+-ATPase, while the vanadyl ion (+4 oxidation state) is a non-inhibitor. Sakurai *et al.*[13] have confirmed by optical and ESR spectrometry that the chloropromazine free radical ion and vanadyl ion are simultaneously formed by the redox reaction between CPZ (**27**) and vanadate ion. The therapeutic effect of CPZ on a vanadate ion-dependent manic depressive illness[14] seems to be due to the formation of the CPZ free radical ion by the redox reaction between vanadate ion and CPZ. Thus, it might be possible to assess the therapeutic action of phenothiazine drugs by measuring the peak heights of vanadyl ion signals formed in the redox reaction between vanadate ion and the drugs, by using ESR spectroscopy.

To test this hypothesis, we measured the ESR spectra of the vanadyl ion signals ($g_0=1.984$, $g_{//}=1.934$, $g_\perp=2.009$, $A_0=11.5$ mT, $A_{//}=20.3$ mT and $A_\perp=7.2$ mT) formed in the redox reaction between vanadate ion and four phenothiazine drugs, **26**—**29**, or five synthesized compounds **32d**, **45**, **46**, **49** and **50**, and/or **25** as a standard Ca^{2+} antagonistic agent. As shown in Table X, the reduction rate in terms of the peak height (mm) of the vanadyl ion signal at gain 2000 was found to be in the following order: **26** > **27** > **28** > **29** > **49** ≫ **45** ≫ **50** > **46** > **25** > **32d**. The following conclusions can be drawn. I) The usual phenothiazine drugs (**26**—**29**) show strong reducing ability towards vanadate ion, but the new-type Ca^{2+} antagonistic agent (**46**) containing the phenothiazine skeleton shows weak reducing ability. These results indicate that new Ca^{2+} antagonistic agents containing the phenothiazine skeleton can be identified by measurement of the IC_{50} value for the inhibition of KCl-induced contractions of rat aorta together with measurement of the peak height of the vanadyl ion signal formed in the redox reaction between vanadate ion and the phenothiazine derivatives by ESR spectroscopy. II) Kielholz[15] has reported that the order of hypnogenic and sedative actions of phenothiazine drugs is **26** > **27** > **28** > **29**, and that the order of antipsychotic effect is **29** > **28** > **27** > **26**. Namely, the strength of the hypnogenic and sedative actions of the phenothiazine drugs is in proportion to that of the reducing ability for vanadate ion of the phenothiazine drugs. These results indicate that the possible therapeutic action of the phenothiazine drugs can be estimated by ESR spectroscopy in terms of reducing ability of vanadate ion by the drugs.

Experimental

Melting points were determined by the capillary method without correction. IR spectra were recorded on a Hitachi 215 infrared spectrophotometer. ^{1}H-NMR spectra were recorded on JEOL PS-100 and JEOL JNM-FX-200 spectrometers using tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a JEOL JMS-D-300 mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on precoated glass plates with Merck Kieselgel $60F_{254}$ as the adsorbent. After development, the plates were air-dried, and exposed to ultraviolet (UV) light and/or sprayed with 1% cerium sulfate in 10% aqueous sulfuric acid, and heated. Preparative thin layer chromatography (P-TLC) was performed on 20×20 cm glass plates coated with Merck Kieselgel $60PF_{254}$ as the adsorbent (20 g per sheet). Column chromatography was performed on Merck Kieselgel 60 (70—230 mesh) and Merck Aluminium oxide 90 Neutral (Al_2O_3). Solvents were purified and dried by standard methods.

Cleavage of Aromatic Methylenedioxy Compounds (1, 4, 5, 8 and 9) with MeONa and EtSH in DMSO A mixture of 6-bromopiperonal (**1**) (100 mg, 0.43 mmol), MeONa (48.8 mg, 0.9 mmol), and EtSH (0.45 ml, 6 mmol) in DMSO (0.5 ml) was stirred at 40 °C for 20 min. The reaction mixture was diluted with cold water (20 ml), NaOH (30 mg) was added, and the whole was extracted with diethyl ether (50 ml×3). The starting material (**1**) (19.1 mg, 19.1%) was recovered from the extract. The mother liquor was acidified (pH 5) with concentrated HCl, and extracted with diethyl ether (70 ml×2). The extract was washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by P-TLC (eluent benzene–acetone, 20:1) to give a 2-bromo-4-ethylthio-5-hydroxybenzaldehyde (**2**) (50.7 mg, 44.5%), mp 119.5—122.5 °C (from benzene).

Compounds **4** and **5** were cleaved similarly (Table I). The cleavage of **8** and **9** under the same conditions gave only non-cleavage products (**10** and **11**, respectively), as shown in Table I. The physical and spectral data of **2**, **6**, **7**, **10** and **11** are given in Tables III—V.

Cleavage of Aromatic Methylenedioxy Compounds (1, 4, 5, 8, 9 and 12) with MeONa and PhSH in DMSO A mixture of **1** (100 mg, 0.43 mmol), MeONa (48.8 mg, 0.9 mmol), and PhSH (0.45 ml, 4.4 mmol) in DMSO (0.5 ml) was stirred at 130 °C for 3 min. The reaction mixture was diluted with cold water (20 ml), NaOH (30 mg) was added, and the whole was extracted with diethyl ether (50 ml × 3). The extract was purified by P-TLC (eluent benzene–acetone, 50 : 1) to give 6-phenylthiopiperonal (**15**) (29.0 mg, 25.7%) and 6-bromopiperonyl alcohol (**16**) (4.3 mg, 4.1%) as non-cleavage products. The mother liquor was acidified (pH 5) with concentrated HCl, and extracted with diethyl ether (70 ml × 2). The extract was washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by P-TLC (eluent benzene–acetone, 30 : 1) to give 2-bromo-4-hydroxy-5-phenylthiomethoxybenzaldehyde (**13**) (59.4 mg, 40.1%), mp 123.0—124.5 °C (from $CHCl_3$–*n*-hexane) and 4-hydroxy-2-phenylthio-5-phenylthiomethoxybenzaldehyde (**14**) (33.3 mg, 20.7%), mp 111.5—114.0 °C (from $CHCl_3$–*n*-hexane) as cleavage products. The cleavage of **4**, **5**, **8**, **9**, and**12** was carried out similarly to give the cleavage compounds **19**, **20**, **14**, **17** and **21**, respectively, as shown in Table II. The physical and spectral data of compounds **14**, **15**, **17**, **18**, **19**, **20** and **21** are shown in Tables III and IV.

Cleavage of 3,4-Methylenedioxynitrobenzene (4) with MeONa and EtSH and EtOH in DMSO A mixture of **4** (75.2 mg, 0.45 mmol), MeONa (97.6 mg, 1.8 mmol), EtSH (0.45 ml, 6 mmol) and EtOH (0.45 ml) in DMSO (0.5 ml) was stirred at 40 °C for 20 min. The reaction mixture was treated with cold water (30 ml), NaOH (30 mg) was added, and the whole was extracted with diethyl ether (50 ml × 3). The extract gave the starting material (**2**) (11.3 mg, 15.0%). The mother liquor was acidified (pH 5) with concentrated HCl and extracted with diethyl ether (70 ml × 2). The extract was washed with water, dried ($MgSO_4$), and concentrated *in vacuo* to give the residue, which was crystallized from benzene–*n*-hexane to give a product (**6**) (81.2 mg, 90.6%), mp 90.0—93.0 °C. Compound **6** was shown to be identical with authentic **6** by direct comparison of the IR and ^{1}H-NMR spectra, and by mixed melting point determination.

Cleavage of 4 with MeONa–PhSH–PhOH in DMSO A mixture of **4** (75.2 mg, 0.45 mmol), MeONa (146.4 mg, 2.7 mmol), PhSH (0.45 ml, 4.4 mmol) and PhOH (0.45 ml) in DMSO (0.5 ml) was stirred at 130 °C for 20 min. The reaction mixture was treated with cold water (30 ml), then acidified (pH 5) with concentrated HCl and extracted with diethyl ether (50 ml × 3). The extract was washed with water, dried ($MgSO_4$) and concentrated *in vacuo* to give the residue, which was purified to give **4** (1.7 mg, 2.3%) from the first fraction and **19** (93.6 mg, 74.9%) from the second fraction by column chromatography (Merck Kieselgel 60, benzene). Compounds **4** and **19** were shown to be identical with the authentic samples **4** and **19** by direct comparison of the IR and ^{1}H-NMR spectra, and mixed melting point determination.

Cleavage of 4 with MeONa and PhSSPh in DMSO A mixture of **4** (75.2 mg, 0.45 mmol), MeONa (73.2 mg, 1.35 mmol) and PhSSPh (900 mg, 4 mmol) in DMSO (0.5 ml) was stirred at 130 °C for 3 min. Work-up in the usual way gave **4** (22.4 mg, 29.8%), **19** (61.7 mg, 49.5%), and **22** (4.4 mg, 7.2%), which were identical with the corresponding authentic samples[1a] by direct comparison of the IR and ^{1}H-NMR spectra, and mixed melting point determination.

Cleavage of 4 with MeONa and Thiols (2b and 2d) in DMSO A mixture of **4** (300.8 mg, 1.8 mmol), MeONa (270 mg, 5 mmol), 2-bromothiophenol (**2b**, 2.0 ml, 15.9 mmol) in DMSO (2.5 ml) was stirred at 140 °C for 60 min. The reaction mixture was poured into cold water (50 ml) and acidified (pH 5) with concentrated HCl. The precipitates were collected by filtration, washed with water, dried, and chromatographed (Merck Kieselgel 60, benzene) to give 2-(2-bromophenylthiomethoxy)-4-nitrophenol (**30a**) (486 mg, 75.8%). The cleavage of **4** with MeONa–2-aminothiophenol (**2d**)–DMSO systems was carried out similarly to give the cleavage compound **30b**, as shown in Table VI. The physical and spectral data of compounds **30a** and **30b** are given in Tables IV and VII.

Cleavage of 9 with MeONa and Thiols (2b, 2c and 2d) in DMSO A mixture of **9** (221.6 mg, 0.9 mmol), MeONa (146.4 mg, 2.7 mmol), and **2b** (1.75 ml, 13.9 mmol) in DMSO (1.0 ml) was stirred at 130 °C for 3 min. The reaction mixture was treated with cold water (50 ml), acidified (pH 5) with concentrated HCl, and extracted with diethyl ether (150 ml × 3). The extract was washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (Merck Kieselgel 60, benzene) to give 5-(2-bromophenylthio)-2-(2-bromophenylthiomethoxy)-4-nitrophenol (**32a**) (394.8 mg, 80.7%), mp 156.0—159.0 °C (from benzene) from the second fraction and 1-(2-bromophenylthio)-4,5-methylenedioxy-2-nitrobenzene (**31a**) (35 mg, 11.0%) from the first fraction.

The cleavage of **9** with the MeONa–2-chlorothiophenol (**2c**) or **2d**–DMSO system was carried out similarly to give the cleavage compounds **32b** and **32c** and non-cleavage compounds **31b** and **31c**, respectively, as shown in Table VI. A mixture of **31c** (52.6 mg, 0.18 mmol), $ClCOCH_2Cl$ (0.5 ml) and anhydrous benzene (4 ml) was stirred at room temperature for 4 h and evaporated *in vacuo*. A mixture of the residue and diethylamine (1.5 ml) in toluene (4 ml) was refluxed for 2 h. The reaction mixture was evaporated in vacuo to give a yellow powder, which was chromatographed (Merck Kieselgel 60, benzene) to give 2′-(4,5-methylenedioxy-2-nitrophenylthio)-2-diethylaminoacetoanilide (**31d**) (69.9 mg, 94.9%). Similarly, compound **32d** was prepared from **32c**. The physical and spectral data of compounds **31d**, **32a**, **32b**, **32c** and **32d** are given in Tables IV, VII and VIII. **31a**: mp 147.0—148.0 °C (from benzene). IR (KBr): 1500, 1300 (NO_2), 930 (methylenedioxy) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.02 (2H, s), 6.08 (2H, s), 7.68 (1H, s). High-resolution MS Calcd for $C_{13}H_8BrNO_4S$:352.9358 and 354.9338. Found: 352.9361 and 354.9338. **31b**: mp 136.5—138.0 °C (from benzene). IR (KBr): 1510, 1310 (NO_2), 930 (methylenedioxy) cm^{-1}. High-resolution MS Calcd for $C_{13}H_8ClNO_4S$: 308.9863 and 310.9833. Found: 308.9861 and 354.9338.

Synthesis of Methyl-Ether Derivatives (33, 34b and 34c) from 30a, 32a and 32b with K_2CO_3–Me_2SO_4 A mixture of **32a** (817 mg, 1.5 mmol), anhydrous K_2CO_3 (10.92 g, 0.08 mol), dimethyl sulfate (7.2 ml) in dry acetone (105 ml) was refluxed for 4 h. After removal of the K_2CO_3 by filtration, the filtrate was concentrated to give a residue that was dissolved in ethyl acetate (AcOEt) (150 ml). The AcOEt solution was washed with 10% NaOH (40 ml × 2) and water (50 ml × 3), and dried over $MgSO_4$. After evaporation of the solvent, the residue was crystallized from *n*-hexane–benzene (1 : 2) to give **34b** in 95% yield.

Similarly, the reaction of compounds **30a** and **32b** with K_2CO_3–Me_2SO_4 gave **33** and **34c** in 85.9 and 90.0% yields, respectively. **33**: mp 99.0—102.0 °C (from *n*-hexane–benzene). IR (KBr): 1520, 1345 (NO_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.90 (3H, s), 5.62 (2H, s), 6.90 (1H, d, J=8.2 Hz), 7.98 (1H, dd, J=8.2, 2.0 Hz). *Anal.* Calcd for $C_{14}H_{12}BrNO_4S$: C, 45.42; H, 3.24; N, 3.78. Found: C, 45.42; H, 3.19; N, 3.72. **34b**: mp 108.0—110.0 °C (from *n*-hexane–benzene). IR (KBr): 1500, 1335 (NO_2) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.48 (3H, s), 5.57 (2H, s), 6.08 (1H, s), 7.88 (1H, s). *Anal.* Calcd for $C_{20}H_{15}Br_2NO_4S_2$: C, 43.11; H, 2.71; N, 2.51. Found: C, 42.96; H, 2.41; N, 2.71. **34c**: mp 126.0—128.0 °C (from *n*-hexane–benzene). IR (KBr): 1510, 1330 (NO_2) cm^{-1}. *Anal.* Calcd for $C_{20}H_{15}Cl_2NO_4S_2$: C, 51.29; H, 3.23; N, 2.99. Found: C, 51.54; H, 3.34; N, 2.87.

Synthesis of 39a, 39b and 40 from 17, 32a and 34b A solution of $NiCl_2 \cdot 6H_2O$ (133.4 mg, 0.52 mmol) in benzene–MeOH (1 : 1, 2 ml) was added to a solution of **34b** (146.0 mg, 0.26 mmol) in benzene (4 ml). To this stirred mixture, $NaBH_4$ (59.3mg, 1.56mmol) was added during 15 min under ice-cooling, and the whole was further stirred at room temperature for 30 min. The reaction mixture was concentrated under reduced pressure, and the residue was chromatographed (Al_2O_3, benzene) to give an amino compound (**36b**, 146.8 mg). A mixture of **36b** (146.8 mg), acetic anhydride (Ac_2O, 1.25 ml) and pyridine (1.25 ml) was stirred for 60 min at room temperature and evaporated *in vacuo*. Purification of the residue by P-TLC (eluent benzene–acetone, 10 : 1) afforded **40** (78.5 mg, 52.6%) from **34b**. **36b**: ^{1}H-NMR ($CDCl_3$) δ: 3.70 (3H, s), 5.57 (2H, s), 6.47 (1H, s), 6.95 (1H, s). **40**: mp 126.0—128.0 °C (from acetone). IR (KBr): 3350, 1680, 1600 (NHCOMe), 1755 (OCOMe) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.05 (3H, s), 3.77 (3H, s), 5.63 (2H, s), 7.10 (1H, s), 8.33 (1H, s). *Anal.* Calcd for $C_{22}H_{19}Br_2NO_3S_2$: C, 46.41; H, 3.36; N, 2.41. Found: C, 46.55; H, 3.35; N, 2.51.

Compounds **39a** and **39b** were prepared from **17** and **32a** by an analogous procedure, as shown in Chart 4. **39a**: mp 115.0—118.0 °C (from benzene–acetone). IR (KBr): 3310, 1680, 1600 (NHCOMe), 1750 (OCOMe) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.03 (6H, s), 5.53 (2H, s), 8.43 (1H, s). High-resolution MS Calcd for $C_{23}H_{21}NO_4S_2$: 374.6089. Found: 374.6091. **39b**: mp 137.0—139.0 °C (from MeOH–*n*-hexane). IR (KBr): 3360, 1690, 1600 (NHCOMe), 1760 (OCOMe) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.10 (3H, s), 2.18 (1H, s), 5.54 (2H, s), 8.44 (1H, s). *Anal.* Calcd for

$C_{23}H_{19}Br_2NO_4S_2$: C, 46.25; H, 3.21; N, 2.34. Found: C, 46.31; H, 3.16; N, 2.13.

Synthesis of 41b from 39b A mixture of **39b** (21.5 mg) and 1 N NaOH (3 ml) in MeOH (2 ml) was stirred at 40 °C for 2 h and evaporated *in vacuo*. The residue was dissolved with water (20 ml), acidified by adding concentrated HCl, and extracted with diethyl ether (20 ml × 2). The extract was washed with water, dried over $MgSO_4$, and evaporated *in vacuo* to give **41b** (17.8 mg, 89.1%). Compound **41b** in MeOH (2 ml) was methylated with diazomethane to give **40** (19.3 mg), which was recrystallized from acetone and shown to be identical with authentic **40** by mixed melting point determination and IR spectral comparison. **41b**: 124.0—126.0 °C (from benzene). The physical and spectral data are shown in Tables VII and VIII.

Synthesis of Diethylaminoacetoamide Derivatives (38a, 38b and 37) from 36a, 36b and 35 A mixture of **36a** (109.6 mg, 0.3 mmol), $ClCOCH_2Cl$ (102 mg, 0.9 mmol) and anhydrous benzene (5 ml) was stirred at room temperature for 30 min and evaporated *in vacuo*. A mixture of the residue and diethylamine (0.2 ml) in anhydrous toluene (2 ml) was refluxed for 5 h. The reaction mixture was evaporated *in vacuo*, and the residue was chromatographed [Merck Kieselgel 60, benzene–$CHCl_3$ (1 : 2)] to give 4′-methoxy-2′-phenylthio-5′-phenylthiomethoxy-2-diethylaminoacetanilide (**38a**, 108.2 mg, 75.5%). Compounds **38b** and **37** were prepared from **36b** and **35** by an analogous precedure, as shown in Chart 4. **38a**: mp 77.5—79.0°C (from benzene–*n*-hexane). IR (KBr): 3450, 1670 (NHCO) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (6H, t, J=7.0 Hz), 2.39 (4H, q, J=7.0 Hz), 3.00 (2H, s), 3.77 (3H, s), 5.57 (2H, s), 8.45 (1H, s). High-resolution MS Calcd for $C_{26}H_{31}N_2O_3S_2$: 482.1698. Found: 482.1720. **38b**: mp 141.5—144.0 °C (from benzene–*n*-hexane). The physical and spectral data are given in Tables VII and VIII. **37**: Brown oil. IR (KBr): 3280, 1680, 1600 (NHCO) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.07 (6H, t, J=7.0 Hz), 2.63 (4H, q, J=7.0 Hz), 3.13 (2H, s), 3.79 (3H, s), 5.60 (2H, s), 6.83 (1H, d, J=8 Hz), 7.08 (1H, dd, J=8.0, 2.0 Hz), 7.54 (1H, dd, J=8.0, 2.0 Hz), 7.80 (1H, dd, J=8.0, 2.0 Hz). High-resolution MS Calcd for $C_{20}H_{25}BrN_2O_3S$: 452.0767 and 454.0751. Found: 452.0745 and 454.0763.

Synthesis of Methylenedioxybenzene Derivatives (43a and 44) from 39a, 41a, 39b and 41b A mixture of **39b** (59.7 mg, 0.1 mmol), anhydrous K_2CO_3 (20 mg, 0.14 mmol), Cu powder (3 mg, 0.05 mmol) and CuBr (3 mg, 0.02 mmol) in nitrobenzene (1 ml) was refluxed for 30 min, and additional Cu powder (7 mg, 0.11 mmol) and CuBr (7 mg, 0.05 mmol) were added to the reaction mixture. The reaction mixture was further refluxed for 5 h, poured into ice-water, and extracted with diethyl ether (50 ml × 3). The extract was washed with water, dried over $MgSO_4$ and evaporated *in vacuo* to give an oily residue, which was chromatographed [Merck Kieselgel 60, benzene–acetone (50 : 1)] to give 10-acetyl-2,3-methylenedioxyphenothiazine (**44**, 24.5 mg, 85.2%). Compound **44** was also prepared from **41b** by an analogous procedure, as shown in Chart 5. Compounds **39a** and **41a** were converted into the same methylenedioxy compound (**43a**) by an analogous procedure, as shown in Chart 5. **43a**: ^{1}H-NMR ($CDCl_3$) δ: 2.02 (3H, s), 5.98 (2H, s), 6.98 (1H, s). **44**: The physical and spectral data are shown in Table VII and ^{1}H-NMR ($CDCl_3$) δ: 2.16 (3H, S), 5.94 (2H, d-like, J=2.0 Hz).

Ullmann Reaction of 41c, 41a or 39a and *o*-Iodotoluene with K_2CO_3–Cu Powder–CuBr A mixture of **41c** (56.2 mg, 0.15 mmol), *o*-iodotoluene (31 mg, 0.14 mmol), anhydrous K_2CO_3 (20 mg, 0.14 mmol), Cu powder (2 mg, 0.03 mmol) and CuBr (3 mg, 0.02 mmol) in nitrobenzene (1 ml) was refluxed for 7.5 h and worked up as described for **44** to give **40a** (12.0 mg, 17.5%). Compound **42** was prepared from **39a** and **41a** by an analogous procedure in 43.8 and 45.1% yields, respectively. **40a**: ^{1}H-NMR ($CDCl_3$) δ: 2.02 (3H, s), 2.40 (3H, s), 3.58 (3H, s), 5.36 (2H, s). High-resolution MS Calcd for $C_{29}H_{27}NO_3S_2$: 501.1432. Found: 501.1430. **42**: ^{1}H-NMR ($CDCl_3$) δ: 2.00 (3H, s), 2.03 (3H, s), 5.98 (2H, s), 7.00 (1H, s). High-resolution MS Calcd for $C_{22}H_{19}NO_3S$: 377.1086. Found: 377.1081.

10-Acetyl-2-(2-bromophenylthiomethoxy)-3-methoxyphenothiazine (45) A mixture of **40** (225 mg, 0.4 mmol), anhydrous K_2CO_3 (20 mg, 0.14 mmol), Cu powder (12.8 mg, 0.20 mmol) and CuBr (12.8 mg, 0.095 mmol) in nitrobenzene (3.5 ml) was refluxed for 2 h, and additional anhydrous K_2CO_3 (9.7 mg, 0.07 mmol), Cu powder (12.8 mg, 0.20 mmol) and CuBr (12.8 mg, 0.095 mmol) were added. The reaction mixture was further refluxed for 4 h and worked up as described for **44** to give **45** (85.8 mg, 44.7%). **45**: The physical and IR spectral data are given in Table VII. ^{1}H-NMR ($CDCl_3$) δ: 2.15 (3H, s), 3.78 (3H, s), 5.57 (2H, s-like).

2-(2-Bromophenylthiomethoxy)-10-(2-diethylaminoacetyl)-3-methoxyphenothiazine (46) $LiAlH_4$ (6.9 mg, 0.18 mmol) in dry diethyl ether (1.2 ml) was added dropwise to a stirred solution of **45** (28.9 mg, 0.06 mmol) in dry diethyl ether (1.2 ml) within 15 min at 0 to 5 °C, and stirring was continued for a further 40 min. The reaction mixture was acidified (pH 5) with 5% HCl, alkalized with 10% Na_2CO_3, and extracted with diethyl ether (25 ml × 3). The extract was washed with water, dried over $MgSO_4$ and evaporated *in vacuo* to give the crude product (22.3 mg). A mixture of the crude product (22.3 mg), $ClCOCH_2Cl$ (11.3 mg) and anhydrous benzene (1.0 ml) was stirred at room temperature for 20 min and evaporated *in vacuo* to give the residue (26.6 mg). A mixture of the residue (26.6 mg) and diethylamine (0.5 ml) in toluene (2 ml) was refluxed for 2 h. The reaction mixture was evaporated *in vacuo* to give the residue, which was purified by P-TLC (eluent $CHCl_3$–acetone, 10 : 1) to give **46** (16.3 mg, 46.0%). **46**: The physical and spectral data are shown in Table VII and ^{1}H-NMR ($CDCl_3$) δ: 0.96 (6H, t, J=7.1 Hz), 2.67 (4H, q, J=7.1 Hz), 3.48 (2H, d-like, J=2.0 Hz), 3.86 (3H, s), 5.58 (2H, s).

10-(2-Diethylaminoacetyl)-3,4-methylenedioxyphenothiazine (47) A mixture of **44** (70 mg, 0.25 mmol) and $LiAlH_4$ (19 mg, 0.5 mmol) in dry diethyl ether (4 ml) was stirred at 0 to 5 °C for 30 min. The reaction mixture was acidified (pH 5) with 5% HCl, alkalized with 10% Na_2CO_3, and extracted with diethyl ether (25 ml × 3). The extract was washed with water, dried over $MgSO_4$ and evaporated *in vacuo* to give the crude product (33.3 mg). A mixture of the crude product (33.3 mg), $ClCOCH_2Cl$ (11.3 mg) and anhydrous benzene (3.0 ml) was stirred at room temperature for 1 h and evaporated *in vacuo* to give the residue. A mixture of the residue and diethylamine (2 ml) in toluene (3 ml) was refluxed for 2 h. The reaction mixture was evaporated *in vacuo* to give the residue, which was purified by P-TLC (eluent $CHCl_3$) to give **47** (29.5 mg, 30.3%). **47**: The physical and spectral data are shown in Table VII and ^{1}H-NMR ($CDCl_3$) δ: 0.96 (6H, t, J=7.1 Hz), 2.64 (4H, q, J=7.1 Hz), 3.45 (2H, d-like, J=2.0 Hz), 5.99 (2H, d-like, J=2.0 Hz), 6.86 (1H, s), 7.06 (1H, s).

10-(2-Diethylaminoacetyl)phenothiazine (50) A mixture of **48** (100 mg, 0.57 mmol) and $ClCOCH_2Cl$ (70 mg) in anhydrous benzene (4 ml) was stirred at room temperature for 1 h and evaporated *in vacuo* to give the residue (**49**). A mixture of **49** and diethylamine (2 ml) in toluene (3 ml) was refluxed for 1 h. The reaction mixture was evaporated *in vacuo* to give the residue, which was purified by P-TLC (eluent $CHCl_3$) to give **50** (131.4 mg, 83.8%). **50**: The physical and IR spectral data are given in Table VII. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (6H, t, J=6.5 Hz), 2.57 (4H, q, J=6.5 Hz), 3.43 (2H, s).

Pharmacological Methods (1) Rat Aorta: Male Wistar rats weighing 170—380 g were stunned by a blow on the head and bled. The thoracic aorta was excised and helically cut into strips about 3 mm in width and 25 mm in length. Aorta strips were suspended in organ baths containing 30 ml of modified Krebs bicarbonate solution under a resting tension of 0.8 g. The solution was maintained at 37±0.5 °C and bubbled with 5% CO_2 in O_2. Isometric contractions were measured using an isometric transducer (Ugo Basile, 7004) and recorded on a one-pen recorder (Tokai Irika, TI-101). Cumulative KCl (10—50 mM) dose–response curves were made in the absence and in the presence of test compounds.

(2) Rabbit Aorta: Male rabbits weighing 2.4—3.7 kg were stunned by a blow on the head and bled. The thoracic aorta was excised and helically cut into strips about 5 mm in width and 30 mm in length. Aorta strips were suspended in organ baths with 30 ml of Krebs–Henseleit solution under a resting tension of 2 g. The solution was maintained at 37±0.5 °C and bubbled with 5% CO_2 in O_2. Isometric contractions were measured by using the method described above for experiment (1). Cumulative noradrenaline (10^{-8}—3×10^{-5} M) dose–response curves were made in the absence and in the presence of test compounds.

(3) Guinea-Pig Ileum: Male guinea-pigs weighing 630—770 g were stunned by a blow on the head and bled. The ileum was excised and suspended in an organ bath containing 30 ml of Tyrode solution under a resting tension of 1g. The solution was maintained at 32±0.5 °C and bubbled with 5% CO_2 in O_2. Isometric contractions were measured by using the method described above for experiment (1). Cumulative histamine (10^{-7}—3×10^{-4} M) dose–response curves were made in the absence and in the presence of test compounds.

(4) Guinea-Pig Atrium: Male guinea-pigs weighing 50—720 g were stunned by a blow on the head and bled. The right and left atria were excized and suspended in an organ bath containing 20 ml of Locke solution. The solution was maintained at 30±0.5 °C and bubbled with 100% O_2. Spontaneous heart contractions and contraction rate were measured using an isometric transducer (Ugo Basile, 7006) and

cardiotachometer. Compounds were dissolved in DMSO and other drugs were dissolved in distilled water. Chloropromazine hydrochloride (**27**) and diltiazem hydrochloride (**23**) were obtained from Sigma Chemical Co., *dl*-noradrenaline hydrochloride was from Nakarai Chemical Co. and histamine dihydrochloride from Wako Pure Chemical Co.

Estimation of Vanadate Reduction of 25—29, 32d, 45, 46, 49 and 50 by ESR Spectrometry ESR spectra were measured with a JEOL FE1XG X-band spectrometer with 100 KHz magnetic field modulation at room temperature and liquid nitrogen temperature (77 K). The magnetic field was calibrated by the splitting of Mn (II) in MgO ($\Delta H_{34} = 8.69$ mT), and g-values were standardized using Li-TCNQ ($g = 2.0025$) as a reference. All reactions were performed in 0.1 M sodium phosphate buffer, pH 7.5. A 0.25 ml aliquot of 1 mM test compound was added to the reaction mixture containing 0.25 ml of 1 mM $NaVO_3$ and 2.0 ml of 0.1 M sodium phosphate buffer, and after 30 min an aliquot of the solution was acidified by the addition of 0.3 ml of 4 M HCl before application to the spectrometer.

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