In Vivo Biological Activity of Antioxidative Aminothiazole Derivatives

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For the development of novel antioxidants having therapeutic utility, a new series of condensed 4- and 5-aminothiazole derivatives has been synthesized using simple methods. Condensed 4-aminothiazoles were prepared by the reaction of α-bromolactams with thioamides in ethanol and 5-aminothiazole derivatives were obtained by the treatment of 3-(acylamino)lactams with a thiating agent such as phosphorus pentasulfide and Lawesson's reagent in pyridine. *In vitro* assay of the condensed 5-aminothiazole derivatives showed them to be potent inhibitors of lipid peroxidation. In order to evaluate these compounds in an *in vivo* system, we devised a simple and reproducible method in which the inhibition of characteristic behaviors induced by spinal injection of $FeCl_2$ was expressed numerically. Compounds having strong *in vitro* activity protected the central nervous system from injury caused by iron-dependent lipid peroxidation. The results suggest that the *in vivo* assay developed in this study should be useful as a screening method for antioxidants and also that condensed 5-aminothiazole derivatives are promising candidates for the treatment of traumatic and ischemic injury of the central nervous system.

Key words thiazoloazepine; thiazolopyridine; iron(II) chloride intrathecal injection; antioxidant; central nervous system

Lipid peroxidation (LP) is an important pathological process in a variety of diseases. As the membranes of neurons are rich in polyunsaturated fatty acid, the central nervous system (CNS) is particularly sensitive to LP. LP in the CNS has been thought to play a pivotal role in tissue damage induced by trauma, cerebral ischemia and subarachnoid hemorrhage.[1] It is reported that some antioxidants have beneficial effects in experimental animal models, and may have potential effects as therapeutic agents.[2–5] It is necessary to devise a simpler *in vivo* method of screening for antioxidant activity in the CNS, because the conventional methods are technically difficult and time-consuming.

Iron is involved in the initiation of oxygen free radicals in all living tissues. Normally endogenous iron exists as a complex with proteins such as hemoglobin or ferritin and does not generate oxygen radicals. However, free iron ion is released after injury and ischemia, or from lysed cells and degraded hemoglobin, and its increased availability enhances tissue damage.[6] Iron that is exogenously applied to the CNS induces LP and elicits seizure and brain edema.[7–12] Although the mechanism of the seizure remains unclear, the initiation of LP and its induction by iron is considered to be an important factor.

In the present study, we have found that injection of $FeCl_2$ into the spinal subarachnoidal space (spinal intrathecal injection; i.t. injection[13]) in mice elicits a characteristic excitatory behavior similar to the epilepsy induced by injection of iron salts into the brain.[7–9] This led us to the idea that $FeCl_2$ i.t. injection could be used as a simple and reproducible *in vivo* screening method for centrally acting antioxidants.

We have also synthesized a new series of antioxidants with pharmacological utility. Condensed 5-aminothiazole derivatives were found to have potent *in vitro* LP-inhibitory activity and to constitute a new class of antioxidants that are complementary to conventional antioxidants, such as vitamins C and E, phenolic compounds such as 3,5-di-*tert*-butyl-4-hydroxytoluene and so on.

Here we describe our novel *in vivo* screening method of antioxidants acting on the central nervous system and its application to evaluate the antioxidative activity of condensed 5-aminothiazole derivatives.

Chemistry

In our laboratories, a new method for the preparation of condensed aminothiazole derivatives has been developed,[14,15] which has opened up an easy route for their synthesis. The principle of this route is to construct a thiazole ring on readily available lactams.

The condensed 5-aminothiazole derivatives **7**—**10** and **13**, having various substituents at the C-2 and N-4 positions, were prepared as shown in Chart 1.[14]

For this approach, 3-(acylamino)lactams **4**—**6** were used as the key intermediates, and they were cyclized by reaction with a thiating agent. The starting 3-aminolactams **1** and **3** were synthesized according to the methods previously reported,[16–21] while DL-α-amino-ε-caprolactam (**2**) is commercially available. 3-(Acylamino)-lactams **4**—**6** were prepared in good yields from the 3-aminolactams **1**—**3** and an acyl chloride (method A) or an acyl imidazolide (method B). For the cyclization reaction of 3-(acylamino)lactams **4**—**6**, phosphorus pentasulfide (P_2S_5) (method C) and Lawesson's reagent (method D) were equally effective and a variety of 3-(acylamino)lactams **4**—**6** provided the expected cyclized derivatives, such as 4,5,6,7-tetrahydrothiazolo[5,4-*b*]pyridines (**7**), 5,6,7,8-tetrahydro-4*H*-thiazolo[5,4-*b*]azepines (**8**) and 4,5,6,7,8,9-hexahydrothiazolo[5,4-*b*]azocines (**9**). The N-4 position of **8** was modified through alkylation and acylation of the secondary amine, resulting in the formation of the *N*-methyl **10a** and *N*-acetyl **10b** derivatives, respectively. Similarly, a bicyclic diamide, 3-(acylamino)-2,3,4,5-tetrahydro-1*H*-[1]-benzazepin-2-one (**12**), derived from 3-amino-2,3,4,5-tetrahydro-1*H*-[1]-benzazepin-2-one (**11**),[22] gave the corresponding tricyclic ami-

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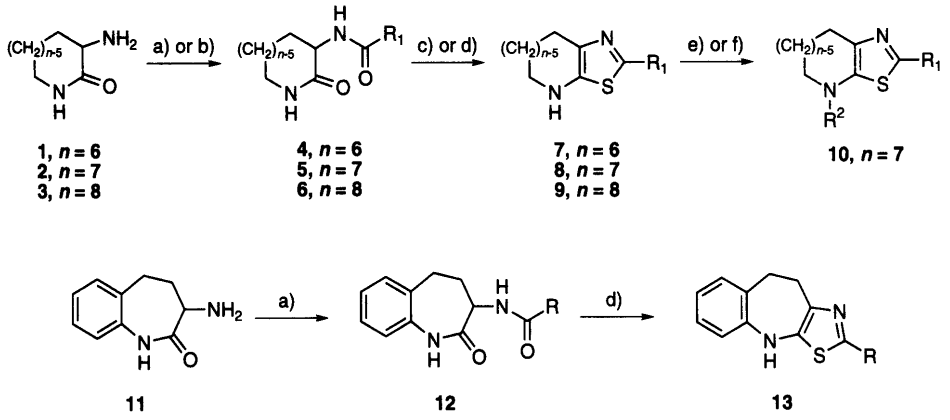
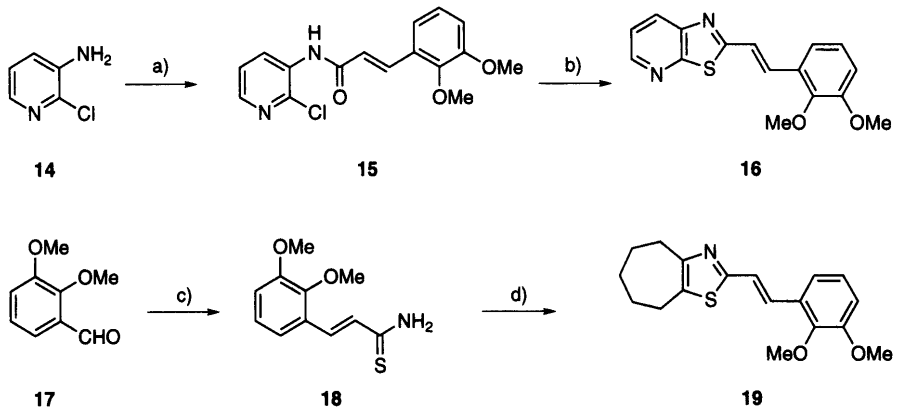


a) R_1COCl, TEA, THF (method A); b) R^1COOH, 1,1'-carbonyldiimidazole, THF (method B); c) P_2S_5, pyridine (method C); d) Lawesson's reagent, pyridine (method D); e) $(MeO)_3PO$, NaH; f) Ac_2O, pyridine.

Chart 1



a) 2,3-Dimethoxycinnamoyl chloride; b) P_2S_5, pyridine (method C); c) thioacetamide, NaOMe, MeOH; d) 2-bromocycloheptanone, EtOH

Chart 2

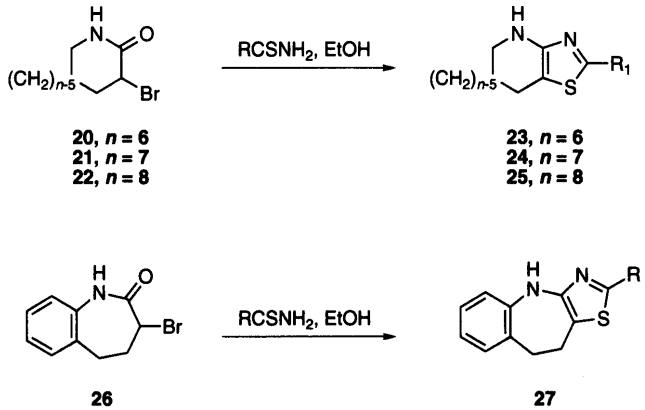


Chart 3

nothiazole **13**.

As shown in Chart 2, the pyridine-fused compound **16**, an analog of **7**, was prepared from **14** using the same procedure as described above for converting **1** to **7**. The cycloheptane analog **19** was prepared by the condensation of 2-bromocycloheptanone with the thioamide **18**[23] in ethanol.

During a series of related studies, we found that the reaction of α-bromolactams **20**—**22** and **26** with the appropriate thioamides in ethanol under reflux produced the corresponding novel condensed bi- and tricyclic 4-aminothiazoles, such as 4,5,6,7-tetrahydrothiazolo[4,5-*b*]pyridines (**23**), 5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*b*]azepines (**24**), 4,5,6,7,8,9-hexahydrothiazolo[4,5-*b*]azocines (**25**) and 9,10-dihydro-4*H*-thiazolo[4,5-*b*][1]benzazepines (**27**), in moderate yields as shown in Chart 3.[15] The structures of all compounds thus prepared were confirmed and characterized by elemental analyses, proton nuclear magnetic resonance (^{1}H-NMR) and mass spectra. Typical procedures for the preparation of these compounds are described in the experimental section.

Biological Results and Discussion

***In Vitro* Lipid Peroxidation Activity** The compounds listed in Table 1 were initially examined for their inhib-

Table 1. Screening Results of Condensed Aminothiazoles

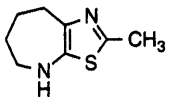
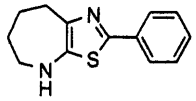
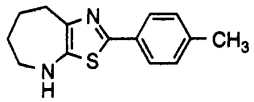
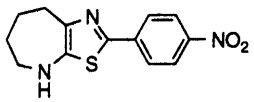
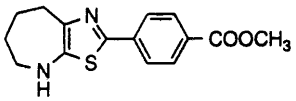
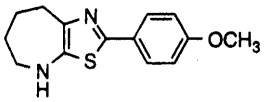
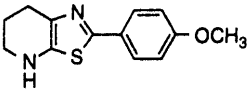
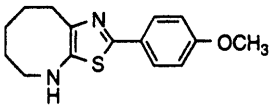
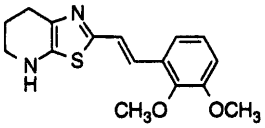
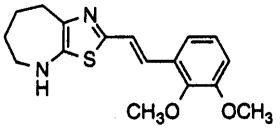
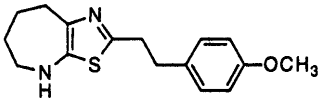
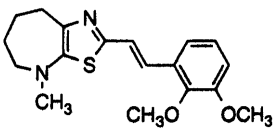
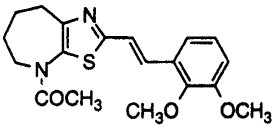
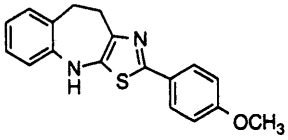
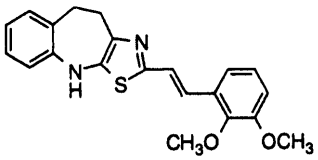
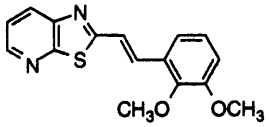
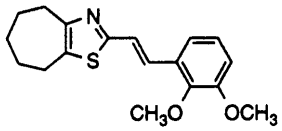
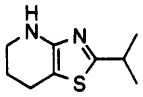
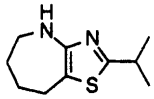
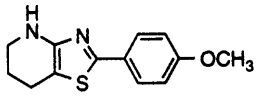
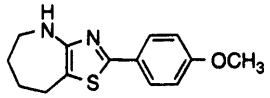
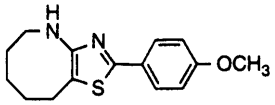
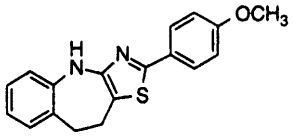
Compd. No.	Brain homogenates[a]		$FeCl_2$ (i.t. method)			
	% (10^{-6} M)	$IC_{50} \times 10^{-6}$ M	0.5 h[b]	1 h[b]	2 h[b]	ID_{50} (mg/kg)[c]
8a 	NT[d]	NT	37.9	12.5	6.1	ND[e]
8b 	71.2	<1	92.9	70.8	22.4	77.4
8c 	97.0	0.53	71.1	14.9	−2.1	ND
8d 	94.0	1.3	57.9	12.2	8.0	ND
8e 	97.0	1.08	35.3	15.6	14.9	ND
8f 	97.2	0.48	92.5	82.0	52.0	58.8
7a 	100	0.19	80.8	73.9	67.4	40.1
9a 	100	0.23	90.3	78.3	79.6	52.9
7b 	97.4	0.81	88.6	82.6	77.1	22.1
8g 	96.9	0.72	89.7	78.3	78.7	28.7
8h 	1.4	3.8	15.8	10.0	8.0	ND
10a 	4.1	ND	63.3	23.3	13.0	ND
10b 	NT	NT	19.4	19.2	12.2	ND

Table 1. (continued)

Compd. No.	Brain homogenates[a]		$FeCl_2$ (i.t. method)			
	% (10^{-6} M)	$IC_{50} \times 10^{-6}$ M	0.5 h[b]	1 h[b]	2 h[b]	ID_{50} (mg/kg)[c]
13a 	95.5	0.41	30.8	7.1	2.3	ND
13b 	98.8	0.63	−28.0	0	0	ND
16 	1.0	ND	21.4	2.2	0	ND
19 	0	ND	−6.7	−11.6	−11.1	ND
23a 	6.6	ND	19.4	15.3	10.2	ND
24a 	2.0	ND	−42.3	10.2	2.0	ND
23b 	31.7	ND	77.8	28.3	−11.4	ND
24b 	8.0	ND	55.2	22.9	20.4	ND
25a 	11.6	ND	41.4	20.8	18.4	ND
27a 	19.7	ND	2.6	4.7	4.8	ND

a) Inhibiting activity on lipid peroxidation in rat brain homogenate. The IC_{50} value of α-tocopherol in brain homogenates was 37.4 at a dose of 100 μM. *b*) Percent inhibition (100 mg/kg, *p.o.*) of the $FeCl_2$-induced responses at each time. *c*) ID_{50} values at 1 h. *d*) NT = not tested. *e*) ND = not determined.

itory activity on LP using rat brain homogenate. After incubation of the homogenate, the resultant LP was evaluated in terms of the formation of thiobarbituric acid products. In this assay, compounds which showed more than 50% inhibition at the concentration of 1 μM were further examined for inhibitory activity on LP at lower concentrations and their IC_{50} values were determined. The results are shown in Table 1.

Substituents at the N-4 position of condensed 5-aminothiazole derivatives remarkably influence the inhibitory activity on LP. Introduction of an alkyl group into the N-4 position (compound **10a**) reduced the activity of the parent compound **8g**, suggesting that a hydrogen atom at the N-4 position is important for potent LP-inhibitory activity.

Regarding the substituent at the C-2 position of 5,6,7,8-

tetrahydro-4*H*-thiazolo[5,4-*b*]azepines, a benzene ring (**8b**) imparted sufficient activity. Introduction of an electron-donating group, such as 4-methyl (**8c**) or 4-methoxy (**8f**), into the phenyl group of **8b** tended to enhance the activity, while electron-withdrawing groups, such as nitro (**8d**) and methoxycarbonyl (**8e**) did not seem to augment the potency. Contraction of the seven-membered ring to a six-membered congener, as in **8f**→**7a**, and ring expansion into an eight-membered ring, as in **8f**→**9a**, caused no loss of activity. In order to examine the effect of the bridge portion in **8b**, insertion of a vinylene unit between the thiazole and benzene ring was carried out. Compounds **7b** and **8g** were prepared and found to exhibit significant activity. However, reduction of the vinylene bridge to ethylene (**8h**) resulted in a considerable decrease in LP-inhibitory activity. Replacement of the caprolactam with a pyridine ring (**7b**→**16**) or with a cycloheptene ring (**8g**→**19**) resulted in a complete loss of activity. Tricyclic derivatives **13a** and **13b** exhibited similar activity to their parent compounds **8f** and **8g**, respectively.

It was particularly interesting to compare the activity of 4-aminothiazole and 5-aminothiazole derivatives. The 4-aminothiazole derivatives **23b**, **24b**, **25a**, and **27a** displayed remarkably lower activities than the corresponding 5-amino congeners, indicating that the free radical-scavenging nature of the N-4 nitrogen atoms of the 5-aminothiazole derivatives is critical for activity.

These results suggested that condensed 5-aminothiazole derivatives having NH at the N-4 position and phenyl or styryl groups at the C-2 position have sufficient antioxidative activity.

***In Vivo* $FeCl_2$-Induced Excitatory Behavior Test** It has been proposed that iron causes neuronal LP with development of an epileptic focus.[8,9] Neuronal LP induced by iron causes disruption of lipids in the neuronal membrane, leading to loss of vital functions, such as mainteinance of the normal ion gradient across the membrane. The decrease in the ion gradients reduces the resting membrane potential, resulting in an increase of neuronal excitability.

It seems reasonable to suppose that the same process occurs in the spinal cord after $FeCl_2$ i.t. injection. First the excitability of the spinal dorsal horn neurons is increased by neuronal LP, which causes excitatory responses such as biting of the lower limbs or abdomen in mice. As LP further progresses to the spinal motoneurons, their excitability is increased, eliciting a series of motor responses, which range from tremor to tonic convulsion according to the severity of the neuronal LP. Complete loss of the ion gradients by neuronal LP causes loss of neuronal function, resulting in paralysis of the lower limbs. At the final stage, almost all the mice die of tonic convulsion of the whole body or paralysis of respiration. On these grounds, we came to the conclusion that the behavioral responses induced by $FeCl_2$ i.t. injection were closely related to LP of the spinal neurons and that the antioxidant activity measured in *in vivo* experiments should be closely related to the potency for protection from behavioral disturbances. Thus, we applied the $FeCl_2$ i.t. model for the *in vivo* screening of centrally acting antioxidants. The results are summarized in Table 1.

Especially in the case of oral administration, *in vivo* results do not necessarily reflect *in vitro* activity because *in vivo* assay is influenced by many other factors such as absorption, distribution, metabolism and excretion. However, in this study a reasonable correlation between *in vitro* and *in vivo* test results was observed for all compounds except the tricyclic derivatives (**13a** and **13b**), whose *in vivo* antioxidative activity was negligible in spite of their strong *in vitro* activity. This difference between *in vitro* and *in vivo* activities is believed to be due to the poor biological availability of **13a** and **13b**. For the bicyclic 5-aminothiazole derivatives, six compounds (**8c**, **8f**, **7a**, **9a**, **7b**, and **8g**) showed antioxidative activity at IC_{50} of less than 1 μM. At 2 h after administration, five of the six compounds inhibited by more than 50% the characteristic behaviors of mice elicited by spinal subarachnoid injection of $FeCl_2$. The five active compounds all have a methoxy group on the benzene ring at the C-2 position, but whether this group works only to enhance the antioxidative activity, or whether it functions in the process of absorption, distribution, metabolism or excretion requires further study.

Conclusions

A series of condensed 5-aminothiazole derivatives obtained by an efficient, versatile and facile route exhibits potent LP-inhibitory activity in rat brain homogenates. In order to evaluate the antioxidative activity in an *in vivo* system, we devised a new assay in which the inhibition of the characteristic behaviors induced by spinal injection of $FeCl_2$ in mice was expressed numerically. In this new assay method, 5-aminothiazole derivatives exhibited strong *in vivo* antioxidative effects, reflecting their *in vitro* activity. Structural elements important for the biological activities are summarized as follows.

(1) For R_1, a conjugated system such as a phenyl or styryl group is important to produce potent antioxidative activity. Compounds bearing an electron-donating group, such as a methoxy group in the benzene ring, are particularly potent.

(2) The hydrogen atom at the N-4 position of the 5-aminothiazoles plays a major role in the antioxidant activity.

(3) 5-Aminothiazole derivatives exhibit more potent activities than the corresponding 4-aminothiazole derivatives. With regard to tricyclic systems, the benzo-fused tricyclic derivatives **13a** and **13b** showed potent *in vitro* activities, but showed negligible activity *in vivo*, perhaps because of low biological availability.

We are now investigating the reason for the great difference in the antioxidative activity of 4- and 5-aminothiazole derivatives.

Experimental

A. Chemistry. General Methods Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Gemini-200 spectrometer in the solvent indicated. Chemical shifts are given in ppm with tetramethylsilane as an internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Reactions were followed by thin-layer

chromatography (TLC) on TLC plates, Silica gel 60 F_{254} precoated (Merck), and chromatographic purifications were carried out on a silica gel column (Kieselgel 60, 0.063—0.200 mm, Merck). Evaporation was carried out *in vacuo* on a rotary evaporator.

Compounds **1**, **3**, **4b**, **5a**, **b**, **d**, **f**, **6a**, **12a**, **20**—**22**, **26**, **27a**, **7b**, **8a**, **b**, **d**, **f**, **9a**, **13a**, **23a**, **b**, **24a**, **b**, **25a**, **27a** were prepared by methods described in the literature[14,15] and were characterized by ^{1}H-NMR and elemental analysis. The data were fully in accordance with the expected structures.

Method A. Preparation of 4-Methoxy-*N*-(2-oxopiperidin-3-yl)benzamide (4a) A solution of 4-methoxybenzoyl chloride (8.97 g, 52.6 mmol) in tetrahydrofuran (THF) (10 ml) was added to an ice-cold solution of 3-amino-2-piperidone (**1**) (5.0 g, 43.8 mmol) and triethylamine (TEA) (5.76 g, 56.9 mmol) in THF (50 ml). The mixture was stirred for 1 h at room temperature, then chloroform ($CHCl_3$) (50 ml) and brine (50 ml) were added. The organic layer was washed with water and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with $CHCl_3$–methanol (MeOH) (95 : 5, v/v). The fractions containing **4a** were combined and concentrated. The residue was recrystallized from ethyl acetate (EtOAc) to yield 8.68 g (80%) of **4a**, mp 183—184 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.50—1.80 (m, 1H), 1.90—2.10 (m, 2H), 2.65—2.80 (m, 1H), 3.38 (t, 2H, J=6.2 Hz), 3.85 (s, 3H), 4.43 (m, 1H), 6.03 (br s, 1H), 6.92 (d, 2H, J=8.8 Hz), 7.11 (br s, 1H), 7.79 (d, 2H, J=8.8 Hz). *Anal.* Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.94; H, 6.48; N, 11.46.

***N*-(2,3,4,5-Tetrahydro-1*H*-2-oxo[1]benzazepin-3-yl)-2,3-dimethoxycinnamamide (12b)** This compound was prepared in a manner similar to that used for **4a** by reacting 3-amino-2,3,4,5-tetrahydro-1*H*-[1]-benzazepin-2-one (**11**)[22] with 2,3-dimethoxycinnamoyl chloride in the presence of TEA in THF in 55% yield, mp 234—236 °C (EtOAc–EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.97—2.42 (m, 2H), 2.60—2.83 (m, 2H), 3.73 (s, 3H), 3.82 (s, 3H), 4.36 (m, 1H), 6.78 (d, 1H, J=15.9 Hz), 7.00—7.35 (m, 7H), 7.58 (d, 1H, J=15.9 Hz), 8.35 (d, 1H, J=8.1 Hz), 9.85 (s, 1H). *Anal.* Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.50; H, 6.05; N, 7.64.

Method B. Preparation of 4-Methyl-*N*-(2-oxoperhydroazepin-3-yl)benzamide (5c) 1,1′-Carbonyldiimidazole (10.5 g, 64.7 mmol) was added to a solution of 4-methylbenzoic acid (8.0 g, 58.8 mmol) in THF (150 ml). The mixture was stirred for 20 min at room temperature, then 3-aminoperhydroazepin-2-one (**3**) (7.53 g, 58.8 mmol) was added. Stirring was continued for 2 h at room temperature, then $CHCl_3$ (50 ml) and brine (50 ml) were added. The organic layer was washed with water and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with $CHCl_3$–MeOH (95 : 5, v/v). The fractions containing **5c** were combined and concentrated. The residue was recrystallized from EtOAc–MeOH to yield 9.8 g (67%) of **5c**, mp 202—203 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.22—1.98 (m, 6H), 2.36 (s, 3H), 3.14 (m, 2H), 4.60 (m, 1H), 7.28 (d, 2H, J=8.0 Hz), 7.76 (d, 2H, J=8.0 Hz). *Anal.* Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.20; H, 7.41; N, 11.12.

The following 3-acylaminolactams were prepared in a manner similar to that used for **5c** with the appropriate carboxylic acid derivative.

Methyl 4-[*N*-(2-Oxoperhydroazepin-3-yl)carbamoyl]benzoate (5e) Yield 84%, mp 226—227 °C (MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.20—2.02 (m, 6H), 3.16 (m, 2H), 3.89 (s, 3H), 4.64 (m, 1H), 7.98 (d, 2H, J=9.0 Hz), 8.06 (d, 2H, J=9.0 Hz). *Anal.* Calcd for $C_{15}H_{18}N_2O_4$: C, 62.06; H, 6.25; N, 9.65. Found: C, 61.95; H, 6.38; N, 9.62.

2,3-Dimethoxy-*N*-(2-oxoperhydroazepin-3-yl)cinnamamide (5g) Yield 60%, mp 187—188 °C (EtOAc–EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.18—1.96 (m, 6H), 3.15 (m, 2H), 3.73 (s, 3H), 3.81 (s, 3H), 4.53 (m, 1H), 6.91 (d, 1H, J=16.0 Hz), 7.00—7.20 (m, 3H), 7.62 (d, 1H, J=16.0 Hz). *Anal.* Calcd for $C_{17}H_{22}N_2O_4$: C, 64.13; H, 6.97; N, 8.80. Found: C, 64.10; H, 6.97; N, 8.72.

3-[2-(4-Methoxyphenyl)ethylamino]azepin-2-one (5h) Yield 79%, mp 185—187 °C (EtOAc–MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.10—1.90 (m, 6H), 2.41 (t, 2H, J=7.9 Hz), 2.73 (t, 2H, J=7.9 Hz), 3.00—3.20 (m, 2H), 3.71 (s, 3H), 4.38 (m, 1H), 6.81 (d, 2H, J=8.4 Hz), 7.12 (d, 2H, J=8.4 Hz), 7.70—7.82 (m, 2H). *Anal.* Calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65. Found: C, 66.19; H, 7.72; N, 9.69.

Method C. Preparation of 5,6,7,8-Tetrahydro-2-*p*-tolyl-4*H*-thiazolo-[5,4-*b*]azepine (8c) Phosphorus pentasulfide (8.73 g) was added to a solution of **5c** (9.67 g, 39.3 mmol) in pyridine (150 ml), and the mixture was heated at 100 °C for 7 h. It was cooled to room temperature, then saturated aqueous sodium hydrogen carbonate solution was added and the product was extracted with $CHCl_3$. The organic layer was washed with water and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with $CHCl_3$–dichloromethane (CH_2Cl_2) (9 : 1, v/v). The fractions containing **8c** were combined and concentrated. The residue was recrystallized from cyclohexane to yield 4.17 g (43%) of **8c**, mp 128—129 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.61—1.89 (m, 4H), 2.35 (s, 3H), 2.91—2.97 (m, 2H), 3.11 (t, 2H, J=5.0 Hz), 7.17 (t, 2H, J=8.0 Hz), 7.65 (t, 2H, J=8.0 Hz). *Anal.* Calcd for $C_{14}H_{16}N_2S$: C, 68.81; H, 6.60; N, 11.46; S, 13.12. Found: C, 68.57; H, 6.60; N, 11.62; S, 13.28.

The following 2-substituted 5,6,7,8-tetrahydro-4*H*-thiazolo[5,4-*b*]azepines were prepared in a manner similar to that used for **8c**.

Methyl 4-(5,6,7,8-Tetrahydro-4*H*-thiazolo[5,4-*b*]azepine-2-yl)benzoate (8e) Yield 57%, mp 168—170 °C (MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.45—1.98 (m, 4H), 2.90—3.02 (m, 2H), 3.07—3.18 (m, 2H), 3.92 (s, 3H), 7.78 (d, 2H, J=9.0 Hz), 8.01 (d, 2H, J=9.0 Hz). *Anal.* Calcd for $C_{15}H_{16}N_2O_2S$: C, 62.48; H, 5.59; N, 9.71; S, 11.12. Found: C, 62.39; H, 5.59; N, 9.87; S, 11.11.

5,6,7,8-Tetrahydro-2-(2,3-dimethoxystyryl)-4*H*-thiazolo[5,4-*b*]azepine (8g) Yield 62%, mp 125—126 °C (cyclohexane). ^{1}H-NMR ($CDCl_3$) δ: 1.50—1.89 (m, 4H), 2.90 (t, 2H, J=6.0 Hz), 3.12 (t, 2H, J=5.0 Hz), 3.85 (s, 3H), 3.88 (s, 3H), 6.84 (dd, 1H, J=2.0, 8.0 Hz), 7.05 (t, 1H, J=8.0 Hz), 7.15 (d, 1H, J=16.0 Hz), 7.16 (dd, 1H, J=2.0, 8.0 Hz), 7.31 (d, 1H, J=16.0 Hz). *Anal.* Calcd for $C_{17}H_{20}N_2O_2S$: C, 64.53; H, 6.37; N, 8.85. Found: C, 64.44; H, 6.30; N, 8.91.

5,6,7,8-Tetrahydro-2-[2-(4-methoxyphenyl)ethyl]-4*H*-thiazolo[5,4-*b*]azepine (8h) Yield 26%, mp 80—83 °C (EtOAc–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.64 (m, 2H), 1.79 (m, 2H), 2.83—3.10 (m, 8H), 3.79 (s, 3H), 6.84 (d, 2H, J=8.6 Hz), 7.14 (d, 2H, J=8.6 Hz). *Anal.* Calcd for $C_{16}H_{20}N_2OS \cdot 0.5H_2O$: C, 64.61; H, 7.12; N, 9.42; S, 10.78. Found: C, 64.86; H, 6.87; N, 9.48; S, 11.02.

Method D. Preparation of 4,5,6,7-Tetrahydro-2-(4-methoxyphenyl)thiazolo[5,4-*b*]pyridine (7a) Lawesson's reagent (14.1 g, 34.9 mmol) was added to a solution of **4a** (8.66 g, 34.9 mmol) in pyridine (70 ml), and the mixture was heated at 100 °C for 4 h. It was cooled to room temperature, then saturated aqueous sodium hydrogen carbonate solution was added and the product was extracted with $CHCl_3$. The organic layer was washed with water and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with $CHCl_3$–MeOH (98 : 2, v/v). The fractions containing **7a** were combined and concentrated. The residue was recrystallized from EtOAc–hexane to yield 4.55 g (53%) of **7a**, mp 116—118 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.90—2.06 (m, 2H), 2.86 (t, 2H, J=6.2 Hz), 3.29 (t, 2H, J=5.1 Hz), 3.83 (s, 3H), 6.89 (d, 2H, J=9.0 Hz), 7.71 (d, 2H, J=9.0 Hz). *Anal.* Calcd for $C_{13}H_{14}N_2OS$: C, 63.39; H, 5.73; N, 11.37; S, 13.02. Found: C, 63.28; H, 5.73; N, 11.38; S, 13.20.

9,10-Dihydro-2-(2,3-dimethoxystyryl)-4*H*-thiazolo[5,4-*b*][1]benzazepine (13b) This compound was prepared from **12b** in a manner similar to that used for **7a** in 70% yield, mp 202—203 °C (EtOAc–EtOH). ^{1}H-NMR ($CDCl_3$) δ: 3.05—3.28 (m, 4H), 3.86 (s, 3H), 3.88 (s, 3H), 6.18 (br s, 1H), 6.77—7.40 (m, 9H). *Anal.* Calcd for $C_{21}H_{20}N_2O_2S$: C, 69.21; H, 5.53; N, 7.69; S, 8.80. Found: C, 69.36; H, 5.64; N, 7.62; S, 8.98.

5,6,7,8-Tetrahydro-2-(2,3-dimethoxystyryl)-4-methyl-4*H*-thiazolo[5,4-*b*]azepine hydrochloride (10a) Sodium hydride (60% in oil, 0.57 g, 14.2 mmol) was added to a solution of **8g** (3.0 g, 9.48 mmol) in trimethyl phosphate (25 g, 0.18 mol) at 0 °C and the mixture was heated at 100 °C for 4 h. It was cooled to room temperature, then water was added, and the reaction mixture was extracted with $CHCl_3$. The organic layer was successively washed with water and brine, and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with hexane–EtOAc (7 : 3, v/v). The fractions containing **10a** were combined and concentrated to give the free base of **10a**. This was converted into the hydrochloride by treatment with an EtOH solution previously saturated with hydrogen chloride gas, followed by recrystallization from diethyl ether–EtOH to yield 1.74 g (48%), mp 170—173 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.59 (m, 2H), 1.83 (m, 2H), 2.90 (t, 2H, J=5.7 Hz), 2.99 (s, 3H), 3.10 (t, 2H, J=5.1 Hz), 3.79 (s, 3H), 3.83 (s, 3H), 7.05 (dd, 1H, J=1.9, 7.8 Hz), 7.11 (t, 1H, J=8.1 Hz), 7.25 (dd, 1H, J=2.2, 7.3 Hz), 7.43 (d, 1H, J=16.5 Hz), 7.55 (d, 1H, J=16.5 Hz). *Anal.* Calcd for $C_{18}H_{22}N_2S \cdot 1.5HCl$: C, 56.14; H, 6.15; Cl, 13.81; N, 7.27; S, 8.32. Found: C, 55.86; H, 6.10; Cl, 13.86; N, 7.26; S, 8.59.

4-Acetyl-5,6,7,8-tetrahydro-2-(2,3-dimethoxystyryl)-4*H*-thiazolo[5,4-*b*]azepine (10b) A solution of acetyl chloride (1.94 g, 24.6 mmol) in THF (10 ml) was added to an ice-cold solution of **8g** (3.0 g, 9.48 mmol)

and 4-dimethylaminopyridine (3.47 g, 28.4 mmol) in THF (50 ml). The mixture was stirred for 12 h at room temperature, then $CHCl_3$ (50 ml) and brine (50 ml) were added. The organic layer was washed with water and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with AcOEt–hexane (1 : 1, v/v). The fractions containing **10b** were combined and concentrated. The residue was recrystallized from EtOAc–hexane to yield 1.94 g (57%) of **10b**, mp 105—107 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.70 (m, 2H), 1.92 (m, 2H), 2.16, 2.31 (s, 3H), 2.95 (m, 2H), 3.70 (m, 2H), 3.86 (s, 3H), 3.90 (s, 3H), 6.85—7.30 (m, 4H), 7.50—7.67 (m, 1H). *Anal.* Calcd for $C_{19}H_{22}N_2O_3S$: C, 63.66; H, 6.19; N, 7.81; S, 8.95. Found: C, 63.48; H, 6.10; N, 7.75; S, 8.82.

***N*-[3-(2-Chloropyridyl)]-2,3-dimethoxycinnamamide (15)** A solution of 2,3-dimethoxycinnamoyl chloride (15.2 g, 67.2 mmol) in THF (50 ml) was added to an ice-cold solution of 3-amino-2-chloropyridine (**14**) (8.64 g, 67.2 mmol) in THF (50 ml). The mixture was stirred for 40 min at room temperature, then $CHCl_3$ (50 ml) and brine (50 ml) were added. The organic layer was washed with water and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with hexane–EtOAc–MeOH (68 : 30 : 2, v/v). The fractions containing **15** were combined and concentrated. The residue was recrystallized from EtOAc–hexane to yield 12.8 g (60%) of **15**, mp 111—113 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.90 (s, 6H), 6.69 (d, 1H, J=15.7 Hz), 6.97 (dd, 1H, J=1.7, 8.0 Hz), 7.09 (t, 1H, J=7.9 Hz), 7.21 (dd, 1H, J=1.7, 7.8 Hz), 7.30 (m, 1H), 7.83 (br s, 1H), 8.08 (d, 1H, J=15.7 Hz), 8.13 (m, 1H), 8.90 (dd, 1H, J=1.7, 8.2 Hz). *Anal.* Calcd for $C_{16}H_{15}ClN_2O_3$: C, 60.29; H, 4.74; Cl, 11.12; N, 8.79. Found: C, 60.00; H, 4.79; Cl, 11.08; N, 8.77.

2-(2,3-Dimethoylstyryl)thiazolo-[5,4-*b*]pyridine (16) Phosphorus pentasulfide (8.8 g) was added to a solution of **15** (12.6 g, 39.5 mmol) in pyridine (100 ml), and the mixture was heated at 100 °C for 2 h. Then it was cooled to room temperature, saturated aqueous sodium hydrogen carbonate solution was added, and the product was extracted with $CHCl_3$–MeOH. The organic layer was washed with water and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with $CHCl_3$. The fractions containing **16** were combined and concentrated. The residue was recrystallized from EtOAc to yield 4.30 g (36%) of **16**, mp 140—142 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.91 (s, 3H), 3.93 (s, 3H), 6.95 (dd, 1H, J=1.5, 8.1 Hz), 7.11 (t, 1H, J=7.9 Hz), 7.26 (dd, 1H, J=1.5, 7.7 Hz), 7.41 (m, 1H), 7.45 (d, 1H, J= 16.4 Hz), 7.88 (d, 1H, J=16.4 Hz), 8.21 (dd, 1H, J=1.5, 8.3 Hz), 8.55 (dd, 1H, J=1.6, 4.7 Hz). *Anal.* Calcd for $C_{16}H_{14}N_2SO_2$: C, 64.41; H, 4.73; N, 9.39; S, 10.75. Found: C, 64.26; H, 4.58; N, 9.37; S, 10.75.

2,3-Dimethoxythiocinnamamide (18) Thioacetamide (11.4 g, 152 mmol) was added to a solution of 2,3-dimethoxybenzaldehyde (25.3 g, 152 mmol) and sodium methoxide (28% solution in MeOH) (36 ml) in MeOH (50 ml). The mixture was stirred for 4 d at 5—10 °C, then $CHCl_3$ (100 ml) and brine (50 ml) were added. The organic layer was washed with water and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with $CHCl_3$–MeOH (98 : 2, v/v). The fractions containing **18** were combined and concentrated. The residue was recrystallized from EtOAc to yield 2.83 g (8.3%) of **18**, mp 158—160 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.87 (s, 3H), 3.88 (s, 3H), 6.94 (dd, 1H, J=1.7, 8.0 Hz), 7.05 (d, 1H, J=15.8 Hz), 7.06 (t, 1H, J=7.9 Hz), 7.17 (dd, 1H, J=1.7, 7.8 Hz), 8.00 (d, 1H, J=15.8 Hz), 8.14 (br s, 1H), 8.39 (br s, 1H). *Anal.* Calcd for $C_{11}H_{13}NO_2S$: C, 59.17; H, 5.87; N, 6.27; S, 14.36. Found: C, 59.14; H, 6.03; N, 6.21; S, 14.30.

2-(2,3-Dimethoxystyryl)-5,6,7,8-tetrahydro-4*H*-cycloheptathiazole (19) A mixture of 2-bromocycloheptanone (3.0 g, 15.9 mmol), TEA (2.5 g, 24.5 mmol) and **18** (2.73 g, 12.2 mmol) in EtOH (10 ml) was refluxed for 6 h. After evaporation of the solvent, the residue was dissolved in a mixture of $CHCl_3$ (50 ml) and water (50 ml). The organic layer was successively washed with water and brine, and dried over magnesium sulfate. The filtrate was concentrated and the residue was purified by column chromatography with hexane–EtOAc (9 : 1, v/v). The fractions containing **19** were combined and concentrated to yield 463 mg (12%) of **19** as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.64—1.96 (m, 6H), 2.82 (t, 2H, J=5.5 Hz), 2.96 (t, 1H), 3.86 (s, 3H), 3.87 (s, 3H), 6.86 (dd, 1H, J=1.4, 8.0 Hz), 7.05 (t, 1H, J=8.0 Hz), 7.15—7.30 (m, 2H), 7.52 (d, 1H, J=16.5 Hz). *Anal.* Calcd for $C_{18}H_{21}NO_2S \cdot 0.5H_2O$: C, 66.64; H, 6.83; N, 4.32; S, 9.88. Found: C, 66.98; H, 7.05; N, 3.94; S, 9.83.

B. Biological Evaluation. Inhibitory Action on Lipid Peroxide Production in Rat Brain Homogenates Lipoperoxide (LPO) produced in the brain homogenate was determined according to the method of Stocks.[24] In brief, brain tissue from male Wistar rats (about 10 weeks of age) was obtained after decapitation and homogenized in ice-cold phosphate-saline buffer (50 mM, pH 7.4). The homogenate was centrifuged for 10 min at 1000 × *g*, and the supernatant was stored at −20 °C until use. The stock brain homogenate was diluted three-fold with the same phosphate-saline buffer. The diluted homogenate (1 ml) was incubated at 37 °C for 30 min with or without the test compound dissolved in 10 μl of dimethyl sulfoxide. The reaction was stopped by adding 200 μl of 35% $HClO_4$, and the mixture was centrifuged at 1300 × *g* for 10 min. The LPO of the supernatant was measured by the thiobarbituric acid method and expressed as malondialdehyde per mg of protein.[25]

***In Vivo* $FeCl_2$ Assay** Male Slc:ICR mice (5 weeks old) were used. $FeCl_2$ was dissolved in saline at a concentration of 50 mM and then injected intrathecally at 5 μl per mouse. Immediately after the i.t. injection, vigorous reciprocal biting or licking of the hind legs and lower abdomen was observed. This immediate excitatory response lasted less than 3 min before disappearing. It was neglected because the solution was acidic and i.t. injection of dilute HCl elicited the same response. About 10 min later, characteristic behavioral response was observed again, and the response was scored at 0.5, 1.0 and 2.0 h after the i.t. injection according to the following criteria:

Score	Behavioral response
0	Normal
1	Repeated biting on the legs or abdomen
2	a) Rigorous biting at the abdomen and sometimes tumbling about, b) Hyperexcitability to external stimuli and aggressiveness or c) Tremor convulsion
3	Clonic convulsion
4	Tonic convulsion or paralysis in one or both legs
5	Death

The test compounds were administered orally 30 min before the $FeCl_2$, i.t. injection, and percent inhibition in each test animal was calculated according to the following formula:

$$\%\ \text{inhibition} = [(\text{the mean score of control group} - \text{the score of the test animal}) / (\text{mean score of control group})] \times 100$$

ID_{50} values and their 95% confidence limits were calculated from the linear regression lines of the log dose–response curves.

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