Synthesis and Pharmacological Activities of Novel Bicyclic Thiazoline Derivatives as Hepatoprotective Agents. I. 8-Ethoxycarbonyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine Derivatives

Masashi HASEGAWA,* Atsushi NAKAYAMA, Toru HOSOKAMI, Yoichi KUREBAYASHI, Takuya IKEDA, Yoshimasa SHIMOTO, Shoichiro IDE, Yuko HONDA, and Norio SUZUKI

Exploratory Research Laboratories III, Daiichi Pharmaceutical Co., Ltd., 16–13, Kitakasai 1-chome, Edogawa-ku, Tokyo 134, Japan. Received July 1, 1994; accepted September 19, 1994

A series of bicyclic thiazoline derivatives (4a—s) was synthesized and evaluated for hepatoprotective activity against galactosamine-induced and monoclonal antibody-induced acute liver injuries in rats. The structure–activity relationships were investigated. Among the compounds synthesized, ethyl 3-(*N*-methylcarbamoyl)-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4p) exhibited remarkable hepatoprotective activity and lower toxicity. This compound suppressed galactosamine-induced hepatic injury at 100 mg/kg by gavage and further prevented monoclonal antibody-induced hepatic injury at 30 mg/kg by intraperitoneal injection, as evaluated by measuring changes in serum transaminase activities.

Keywords ethyl 5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate; bicyclic thiazoline; hepatoprotective agent; galactosamine-induced hepatic injury; monoclonal antibody-induced hepatic injury; structure–activity relationship

It is generally believed that immunological and inflammatory responses at hepatocyte membranes play a major role in the development of liver cell damage in acute and chronic liver diseases including hepatitis B. In order to find an effective hepatoprotective agent, we selected two experimental hepatic injury models in rats. One is galactosamine-induced hepatic injury, which is thought to involve inflammatory reaction, because infiltration of macrophages and neutrophils can be observed histologically.[1] The other is acute liver necrosis induced by a monoclonal IgM antibody to a liver-specific antigen.[2] This reflects the damage caused by complement-mediated immune attack on hepatocytes. We examined a number of bicyclic thiazoline derivatives in these two models to search for a new hepatoprotective agent, using changes in transaminase activities as biological indices. This paper deals with the syntheses, hepatoprotective activities and structure–activity relationships of various 8-ethoxycarbonyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine derivatives **4**.

Molecular Design To our knowledge, no lead compound having potent inhibitory activity in the above two animal models has ever been reported. Piprozolin **1**[3] and malotilate **2**[4] were reported previously to possess hepatoprotective activity against only galactosamine-induced hepatic injury. We assumed that chemical modification of these two compounds might be an effective approach. These compounds both contain a β-thioacrylate moiety, and **1** was reported[3] to take the *Z*-form selectively (Chart 1). Therefore, a conjugated cisoid system between the sulfur atom and the ethoxycarbonyl group is thought to be important for hepatoprotective activity. To fix the configuration between the sulfur atom and the ethoxycarbonyl group of **1** and to incorporate the conjugated system of **2** into the skeleton of **1**, we replaced the thiazoline ring system of **1** with a bicyclic ring system and designed bicyclic thiazoline derivatives **3** as target compound. We selected the sulfur atom as a linker (*Z*) in compound **3** because many hepatoprotective agents possess plural sulfur atoms.[5] To our knowledge, compounds such as **4** have not previously been synthesized.

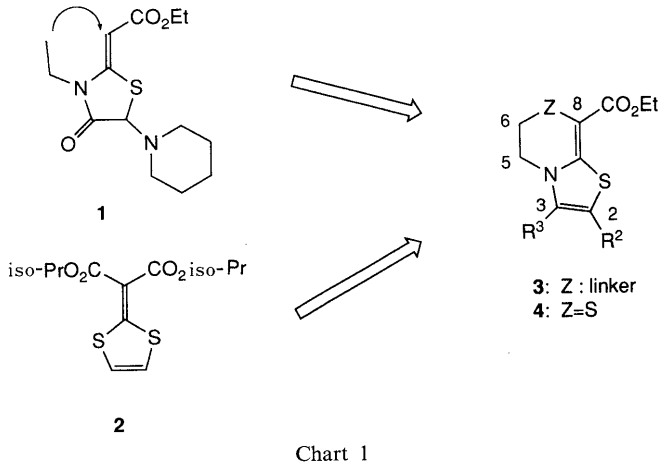

Chart 1

Synthesis The bicyclic thiazolines, 8-ethoxycarbonyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine derivatives **4**, were synthesized from the thiolactam **6** by three methods as outlined in Chart 2. The key intermediate **6** was prepared by treatment of the known lactam **5**[6] with Lawesson's reagent.[7] The first approach, method A, utilized the condensation of **6** with α-haloaldehydes or ketones in acetic acid.[8] The second approach, method B, involved the reaction of **6** with 2,2-dihaloalcohols in the presence of potassium hydroxide in ethanol and subsequent dehydration catalyzed by acetic acid.[9] The third approach, method C, utilized the condensation of **6** with α-haloacyl halide to produce 3-oxo derivatives **7**,[10] which were then converted to the bicyclic thiazolines **4** by reduction with lithium aluminum hydride ($LiAlH_4$) followed by dehydration catalyzed by *p*-toluenesulfonic acid (TsOH).

The preparation of the hydroxyalkyl or carbamoyl derivatives of bicyclic thiazolines is outlined in Chart 3. 3-Hydroxymethyl and hydroxyethyl derivatives (**4r**, **s**) were obtained from the corresponding ethyl esters (**4j**, **k**)

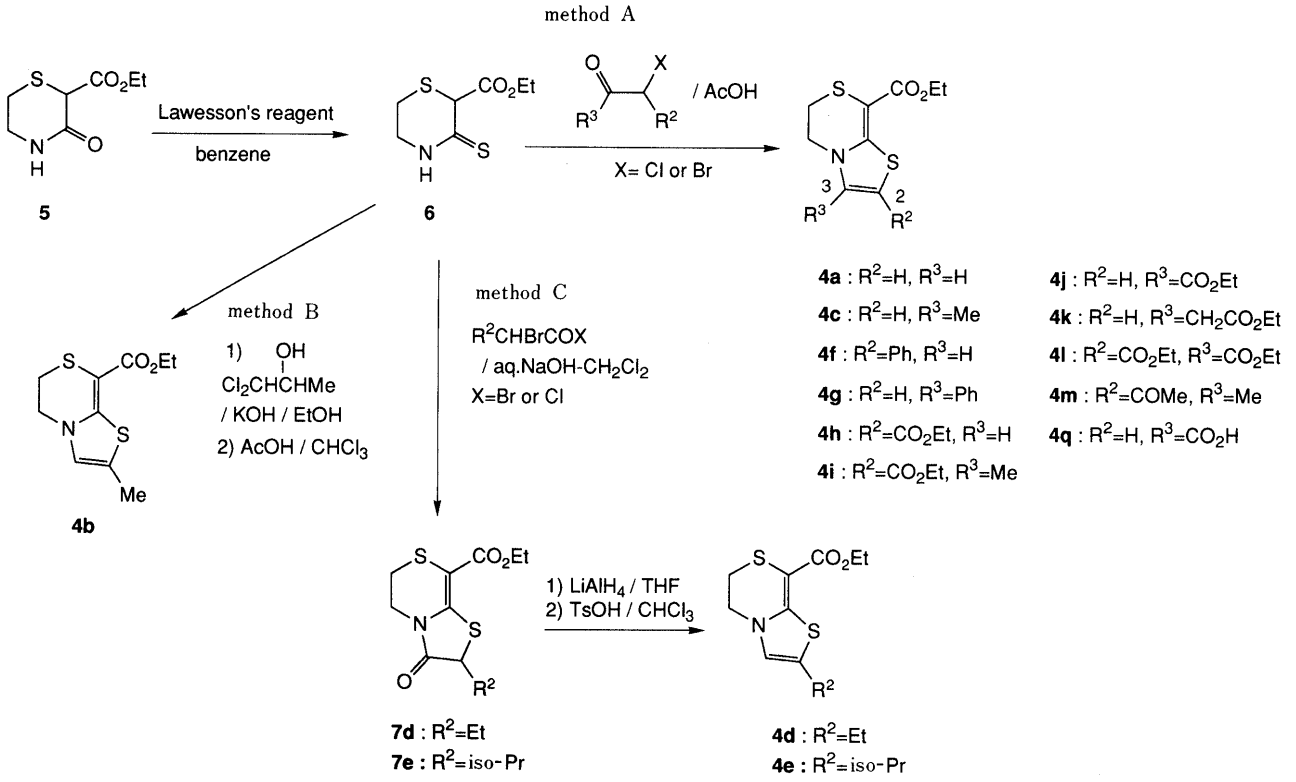

Chart 2

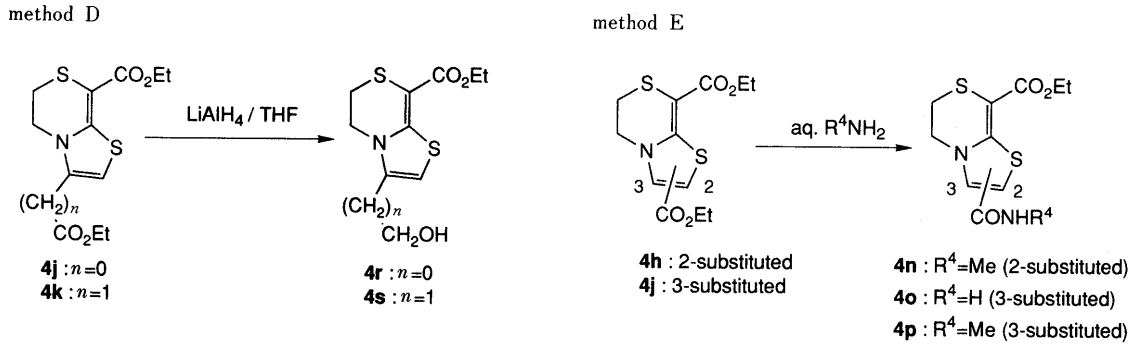

Chart 3

by $LiAlH_4$ reduction. Amidation of the diesters (**4h, j**) with excess amine gave 2- and 3- carbamoyl derivatives (**4n—p**) without formation of diamides. Physicochemical data of the synthesized compounds are listed in Table I.

Pharmacological Evaluation As it is hard to prepare monoclonal antibody on a large scale, the synthesized compounds were first tested for the hepatoprotective activity against galactosamine-induced liver damage in rats[1] by oral administration (100 mg/kg and 300 mg/kg) according to the method described in Experimental. The hepatoprotective activities were evaluated in terms of specific inhibitory rate (%) for the increase in serum glutamic pyruvic transaminase (GPT) activity, and the results are included in Table I.

Nine compounds (**4a, b, d, e, f, h, j, m, p**) which exhibited significant inhibitory activities in the test described above ($p<0.05$) were secondly evaluated by using the monoclonal antibody-induced acute hepatitis model[2] in rats by intraperitoneal administration at 30 mg/kg (see Experimental). As the monoclonal antibody administration induces acute hepatitis within 1 h, the nine compounds processed in the second series of evaluation assays were administered intraperitoneally. The hepatoprotective activities were also evaluated in terms of specific inhibitory rate (%) for the increase in serum GPT activity, and the results are shown in Table II.

Structure–Activity Relationships Compound **4a**, bearing no substituent on the bicyclic thiazoline ring except for an ethoxycarbonyl group at position 8, showed protective activity against hepatocellular damage evoked by galactosamine or monoclonal antibody. With the aim of generating more effective compounds, several substituents were introduced into the 2- or 3-position of **4a**. We examined the activities of the bicyclic thiazolines **4b—g** having an alkyl or a phenyl group. Among these compounds, only **4b** having a methyl group at the 2-position was active in both models. But compounds having a longer alkyl or phenyl group at the 2-position

TABLE I. Derivatives of Ethyl 5,6-Dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate **4** and Their Hepatoprotective Activities against Galactosamine-Induced Hepatitis (*p.o.*)

Compound No.	R^2	R^3	Method[a] (Yield (%))	mp (°C)	Formula	Analysis (%) Calcd (Found)			Hepatoprotective activity (% inhibition)[b]	
						C	H	N	100 mg/kg	300 mg/kg
4a	H	H	A (40)	86	$C_9H_{11}NO_2S_2$	47.14 (46.89)	4.84 (4.67)	6.11 (5.94)	76[f]	86[f]
4b	Me	H	B (36)	142—143	$C_{10}H_{13}NO_2S_2$	49.36 (49.35)	5.38 (5.35)	5.76 (5.74)	67	78[f]
4c	H	Me	A (47)	129—130	$C_{10}H_{13}NO_2S_2$	49.36 (49.03)	5.38 (5.30)	5.76 (5.83)	72	69
4d	Et	H	C (42)[c]	127—128	$C_{11}H_{15}NO_2S_2$	51.34 (51.44)	5.87 (5.93)	5.44 (5.52)	13	70[f]
4e	iso-Pr	H	C (50)[c]	77—78	$C_{12}H_{17}NO_2S_2$	53.11 (53.30)	6.31 (6.25)	5.16 (5.24)	52[f]	53[f]
4f	Ph	H	A (30)	152—153	$C_{15}H_{15}NO_2S_2$	58.99 (58.88)	4.95 (5.01)	4.59 (4.58)	87[f]	66[f]
4g	H	Ph	A (68)	156—157	$C_{15}H_{15}NO_2S_2$	58.99 (58.73)	4.95 (4.80)	4.59 (4.65)	39	41
4h	CO_2Et	H	A (24)	195—196	$C_{12}H_{15}NO_4S_2$	47.82 (47.65)	5.02 (4.84)	4.65 (4.71)	94[e]	89[e]
4i	CO_2Et	Me	A (40)	223—225	$C_{13}H_{17}NO_4S_2$	49.51 (49.24)	5.43 (5.23)	4.44 (4.43)	5	45
4j	H	CO_2Et	A (30)	132—133	$C_{12}H_{15}NO_4S_2$	47.82 (47.61)	5.02 (4.83)	4.65 (4.66)	28	65[f]
4k	H	CH_2CO_2Et	A (39)	95—97	$C_{13}H_{17}NO_4S_2$	49.51 (49.51)	5.43 (5.39)	4.44 (4.47)	−1	59
4l	CO_2Et	CO_2Et	A (32)	106—107	$C_{15}H_{19}NO_6S_2$	48.24 (48.05)	5.13 (4.98)	3.75 (3.79)	11	67
4m	COMe	Me	A (43)	184—185	$C_{12}H_{15}NO_3S_2$	50.51 (50.63)	5.30 (5.31)	4.91 (5.16)	24	60[e]
4n	CONHMe	H	D (72)[d]	215—217	$C_{11}H_{14}N_2O_3S_2$	46.14 (45.81)	4.93 (5.08)	9.78 (9.86)	28	26
4o	H	$CONH_2$	D (37)[d]	206—207	$C_{10}H_{12}N_2O_3S_2$	44.10 (44.09)	4.44 (4.37)	10.29 (10.06)	13	45
4p	H	CONHMe	D (53)[d]	197—198	$C_{11}H_{14}N_2O_3S_2$	46.14 (46.37)	4.93 (4.88)	9.78 (9.84)	73[f]	68[f]
4q	H	CO_2H	A (44)	188—190	$C_{10}H_{11}NO_4S_2$	43.94 (43.88)	4.06 (4.02)	5.12 (4.98)	68	40
4r	H	CH_2OH	E (62)[d]	169—170	$C_{10}H_{13}NO_3S_2$	46.31 (46.33)	5.05 (5.14)	5.40 (5.54)	−65	27
4s	H	$(CH_2)_2OH$	E (29)[d]	134—136	$C_{11}H_{15}NO_3S_2$	48.33 (48.15)	5.53 (5.39)	5.12 (5.14)	−19	33

a) See text. *b*) Protective activities against galactosamine-induced hepatitis in rats ($n=6$) (see Experimental). *c*) Total yield from **6**. *d*) Yield from the corresponding ethyl ester. *e*) $p<0.05$ *vs.* control. *f*) $p<0.01$ *vs.* control.

TABLE II. Hepatoprotective Activities against Monoclonal Antibody-Induced Hepatitis in Rats ($n=5$)

Compound No.	% inhibition[a]
	30 mg/kg i.p.
4a	60[c]
4b	45[b]
4d	20
4e	22
4f	5
4h	39[b]
4j	6
4m	49[b]
4p	71[b]

a) Suppression (%) of the elevation of GPT activity (see Experimental). *b*) $p<0.05$ *vs.* control. *c*) $p<0.01$ *vs.* control.

showed very weak activities in the monoclonal antibody-induced hepatitis model. Substitution of hydrogen at the 2- or 3-position of **4a** with an alkyl or a phenyl group tends to lower the activity. Introduction of a hydroxymethyl or hydroxyethyl group also reduced the activity (**4r**, **s**).

As malotilate **2** has two alkoxycarbonyl groups, we next examined the activities of the bicyclic thiazolines **4h—k** having two ethoxycarbonyl groups. Among the compounds examined, **4h** possessing a 2-ethoxycarbonyl group showed protective activity in both hepatitis models. It is noteworthy that the activity of **4j** against galactosamine-induced hepatitis was more potent than that of the methylene analogue **4k**. Thus, it was presumed that the presence of a carbonyl group adjacent to the thiazole ring was favorable for show hepatoprotective activity. The ethoxycarbonyl, acetyl, carbamoyl and carboxyl derivatives were also prepared (**4l—q**). Among these derivatives, **4p**, bearing the *N*-methylcarbamoyl group at the 3-position, was the most potent compound. A comparison of **4n** and **4p** indicates that the *N*-methylcarbamoyl moiety at the 3-position is essential for hepatoprotective activity. As the methylene analogue of **4p** (3-CH_2CONHMe

derivative) showed no activity (−2%) in the monoclonal antibody-induced hepatitis model in a preliminary study (data are not shown), it was presumed that the presence of the *N*-methylcarbamoyl group adjacent to the thiazole ring, especially at the 3-position, is necessary for hepatoprotective activity.

An acute toxicity study of two of the effective compounds, **4a** and **4p**, was also carried out in mice (1—3 g/kg *p.o.*). The LD_{50} value of **4a** was less than 1 g/kg. In the case of **4p**, no acute toxicological sign was observed even at the dose of 3 g/kg. In conclusion, the novel compound **4p**, which exhibited potent hepatoprotective activity in both models, and low toxicity, was selected for further development as a new hepatoprotective agent.

Experimental

Chemistry Melting points were determined with a Yanagimoto micro melting point apparatus, and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM-FX-90Q spectrometer using dimethylsulfoxide-d_6 (DMSO-d_6) or $CDCl_3$ as a solvent and Me_4Si as an internal standard. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublets) and m (multiplet). Chemical shifts are expressed in δ (ppm) values and the coupling constants are expressed in hertz (Hz). Infrared (IR) spectra were recorded on a Hitachi 260-30 spectrometer using KBr disks. Elemental analyses were performed on a Perkin-Elmer Model 240C elemental analyzer. The yields, melting points and elemental analysis data of **4a**—**s** are given in Table I.

2-Ethoxycarbonyl-5,6-dihydro-2*H*-[1,4]thiazin-3-thione (5) Lawesson's reagent[7] (480 g, 2.54 mol) was added to a stirred solution of **5**[6] (539 g, 2.67 mol) in benzene (3.5 l), and the resulting mixture was stirred at 60—70 °C for 2 h, then allowed to cool to room temperature. Insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was taken up in a mixture of benzene and Et_2O and left to solidify. The resulting solid was collected and dried to give **6** (364 g, 70%) as a pale yellow powder, which was used for the subsequent reaction without further purification. An analytical sample was obtained as follows; the solid was purified by column chromatography on silica gel with a mixture of AcOEt and $CHCl_3$ (1 : 1). The eluate was evaporated and the residual solid was recrystallized from Et_2O–hexane to give pale yellow crystals, mp 97—99 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.16 (3H, t, J=7 Hz), 2.55—3.45 (2H, m), 3.50—3.80 (2H, m), 4.12 (2H, q, J=7 Hz), 4.23 (1H, s), 8.95 (1H, br s). *Anal.* Calcd for $C_7H_{11}NO_2S_2$: C, 40.95; H, 5.40; N, 6.82. Found: C, 40.83; H, 5.32; N, 6.67.

Method A. Ethyl 5,6-Dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4a) An aqueous solution of chloroacetaldehyde (40%, 2.05 g, 10 mmol) was added to a solution of the thiolactam **6** (2.00 g, 9.8 mmol) in acetic acid (40 ml), and the mixture was heated at 50—60 °C for 2 h. After cooling, the reaction mixture was concentrated *in vacuo*. The residue was poured into aqueous 5% Na_2CO_3 and extracted with $CHCl_3$. The extract was washed with water and brine, and dried (Na_2SO_4). After removal of the solvent, the residue was chromatographed on silica gel with $CHCl_3$. The eluate was evaporated and the solid obtained was recrystallized from a mixture of Et_2O and isopropyl alcohol to give 0.89 g (40%) of **4a** as pale yellow crystals. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7 Hz), 2.7—3.0 (2H, m), 3.9—4.2 (2H, m), 4.22 (2H, q, J=7 Hz), 6.26 (1H, d, J=4 Hz), 6.58 (1H, d, J=4 Hz).

Ethyl 3-Methyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4c) The title compound was prepared by the reaction of **6** (1.5 g, 7.3 mmol) with chloroacetone (0.80 g, 8.6 mmol) in the same manner as described above. Pale yellow crystals (0.89 g, 47%, recrystallized from CH_2Cl_2–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, t, J=7 Hz), 2.19 (3H, s), 2.9–3.1 (2H, m), 4.1–4.3 (2H, m), 4.28 (2H, q, J=7 Hz), 6.08 (1H, s).

Ethyl 2-Phenyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4f) The title compound was prepared by the reaction of **6** (1.7 g, 8.3 mmol) with 2-chloro-2-phenylethanal[11] (1.7 g, 11 mmol) in the same manner as described above. Yellow crystals (0.77 g, 30%, recrystallized from benzene). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 2.9—3.1 (2H, m), 4.1—4.4 (2H, m), 4.32 (2H, q, J=7 Hz), 6.92 (1H, s), 7.3—7.6 (5H, m).

Ethyl 3-Phenyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4g) The title compound was prepared by the reaction of **6** (1.5 g, 7.3 mmol) with phenacyl bromide (1.7 g, 8.6 mmol) in the same manner as described above. Yellow crystals (1.52 g, 68%, recrystallized from CH_2Cl_2–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7 Hz), 2.7—3.0 (2H, m), 3.9—4.2 (2H, m), 4.28 (2H, q, J=7 Hz), 6.21 (1H, s), 7.3—7.6 (5H, m).

Diethyl 5,6-Dihydrothiazolo[2,3-*c*][1,4]thiazine-2,8-dicarboxylate (4h) The title compound was prepared by the reaction of **6** (1.7 g, 8.3 mmol) with ethyl 2-chloro-3-oxopropionate[12] (2.4 g, 13 mmol) in the same manner as described above. Yellow crystals (0.61 g, 24%, recrystallized from benzene). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 1.37 (3H, t, J=7 Hz), 2.8—3.1 (2H, m), 4.0—4.3 (2H, m), 4.28 (4H, q, J=7 Hz), 7.37 (1H, s).

Diethyl 3-Methyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-2,8-dicarboxylate (4i) The title compound was prepared by the reaction of **6** (2.2 g, 11 mmol) with ethyl 2-chloroacetoacetate (2.3 g, 14 mmol) in the same manner as described above. Pale yellow crystals (1.38 g, 40%, recrystallized from benzene–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, t, J=7 Hz), 1.36 (3H, t, J=7 Hz), 2.59 (3H, s), 2.9—3.2 (2H, m), 4.1—4.4 (2H, m), 4.30 (2H, q, J=7 Hz), 4.32 (2H, q, J=7 Hz).

Diethyl 5,6-Dihydrothiazolo[2,3-*c*][1,4]thiazine-3,8-dicarboxylate (4j) The title compound was prepared by the reaction of **6** (1.5 g, 7.3 mmol) with ethyl bromopyruvate (1.7 g, 8.5 mmol) in the same manner as described above. Yellow crystals (0.67 g, 30%, recrystallized from CH_2Cl_2–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, t, J=7 Hz), 1.36 (3H, t, J=7 Hz), 2.8—3.0 (2H, m), 4.26 (2H, q, J=7 Hz), 4.30 (2H, q, J=7 Hz), 4.5—4.7 (2H, m), 7.30 (1H, s).

Ethyl (8-Ethoxycarbonyl-5,6-dihydro[2,3-*c*][1,4]thiazin-3-yl)acetate (4k) The title compound was prepared by the reaction of **6** (1.6 g, 7.8 mmol) with ethyl 4-chloroacetoacetate (1.7 g, 9.8 mmol) in the same manner as described above. Yellow crystals (0.95 g, 39%, recrystallized from benzene–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7 Hz), 1.36 (3H, t, J=7 Hz), 2.8—3.1 (2H, m), 3.52 (2H, s), 4.0—4.25 (2H, m), 4.27 (2H, q, J=7 Hz), 4.28 (2H, q, J=7 Hz), 6.16 (1H, s).

Triethyl 5,6-Dihydrothiazolo[2,3-*c*][1,4]thiazine-2,3,8-tricarboxylate (4l) The title compound was prepared by the reaction of **6** (1.7 g, 8.3 mmol) with ethyl β-chloro-β-(ethoxycarbonyl)pyruvate[13] (2.8 g, 12.6 mmol) in the same manner as described above. Orange crystals (1.0 g, 32%, recrystallized from benzene–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.52 (9H, m), 2.9—3.2 (2H, m), 4.1—4.6 (8H, m).

Ethyl 2-Acetyl-3-methyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4m) The title compound was prepared by the reaction of **6** (1.7 g, 8.3 mmol) with ethyl 3-chloro-2,4-pentanedione (1.6 g, 12 mmol) in the same manner as described above. Yellow crystals (0.95 g, 39%, recrystallized from $CHCl_3$–Et_2O). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 2.37 (3H, s), 2.58 (3H, s), 2.9—3.2 (2H, m), 3.0—3.3 (2H, m), 4.30 (2H, q, J=7 Hz).

8-Ethoxycarbonyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-3-carboxylic Acid (4q) Bromopyruvic acid (1.7 g, 10 mmol) was added to a solution of the thiolactam **6** (1.6 g, 9.8 mmol) in acetic acid (10 ml) and the mixture was heated at 50 °C for 1 min. After cooling, the reaction mixture was stirred at room temperature for 30 min to give a precipitate, which was collected and dissolved in AcOEt. The solution was washed with water and brine, and dried (Na_2SO_4). After removal of the solvent, the solid was recrystallized from EtOH to give 0.94 g (44%) of **4q** as yellow crystals. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7 Hz), 2.75—3.05 (2H, m), 3.4—3.7 (2H, m), 4.18 (2H, q, J=7 Hz), 7.42 (1H, s).

Method B. Ethyl 2-Methyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4b) The thiolactam **6** (4.00 g, 19.5 mmol) was added to a solution of potassium hydroxide (85%, 4.00 g, 60.7 mmol) in ethanol (300 ml). 1,1-Dichloro-2-propanol[14] (4.00 g, 31.0 mmol) was added to the mixture at 60—70 °C, and the whole was stirred at the same temperature for 10 min, then allowed to cool. Insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (300 ml) and the solution was washed with water and brine, and dried (Na_2SO_4). Acetic acid (5 ml) was added to the solution and the mixture was stirred at 50 °C for 20 min. After cooling, the solution was washed successively with aqueous 5% Na_2CO_3, water and brine, and dried (Na_2SO_4). Removal of the solvent afforded a residue, which was chromatographed on silica gel with a mixture of AcOEt and hexane (2 : 3). The eluate was evaporated and the resulting solid was recrystallized from CH_2Cl_2–hexane to give 1.70 g (36%) of **4b** as pale yellow crystals. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7 Hz), 2.15 (3H, s), 2.8—3.0 (2H, m), 4.0—4.2 (2H, m), 4.25 (2H, q, J=7 Hz), 6.28 (1H, s).

Method C. Ethyl 2-Ethyl-3-oxo-2,3,5,6-tetrahydrothiazolo-[2,3-*c*]-

[1,4]thiazine-8-carboxylate (7d) A solution of the thiolactam **6** (2.10 g, 10.2 mmol) in CH_2Cl_2 (50 ml) was treated with 0.5 N NaOH (20 ml) and the mixture was stirred vigorously at 0—5 °C. 2-Bromobutanoyl bromide (2.86 g, 12.4 mmol) was added dropwise and the whole was stirred at the same temperature for 15 min. Then 0.5 N NaOH (30 ml) was added at the same temperature. The mixture was stirred at room temperature for 1 h and CH_2Cl_2 (200 ml) was added. The organic layer was separated and washed with water and brine, and dried (Na_2SO_4). After removal of the solvent, the residual crystals were recrystallized from $CHCl_3$–Et_2O to give 2.01 g (72%) of **7d** as colorless crystals, mp 110—111 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (3H, t, J=7 Hz), 1.36 (3H, t, J=7 Hz), 1.5—2.3 (2H, m), 2.9—3.1 (2H, m), 3.90 (1H, dd, J=8 Hz, 5 Hz), 3.95—4.15 (2H, m), 4.30 (2H, q, J=7 Hz). IR: 1712 cm^{-1}, 1674 cm^{-1}. *Anal.* Calcd for $C_{11}H_{15}NO_3S_2$: C, 48.33; H, 5.53; N, 5.12. Found: C, 48.38; H, 5.61; N, 5.26.

Ethyl 2-Isopropyl-3-oxo-2,3,5,6-tetrahydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (7e) The title compound was prepared by the reaction of **6** (3.00 g, 14.4 mmol) with 2-bromo-3-methylbutanoyl chloride[15] (3.21 g, 16.1 mmol) in the same manner as described above. Colorless crystals (3.69 g, 88%, recrystallized from $CHCl_3$–Et_2O). ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, d), 1.08 (3H, d), 1.36 (3H, t, J=7 Hz), 2.3—2.7 (2H, m), 2.9—3.1 (2H, m), 3.9—4.1 (3H, m), 4.30 (2H, q, J=7 Hz). IR: 1704 cm^{-1}, 1678 cm^{-1}. *Anal.* Calcd for $C_{12}H_{17}NO_3S_2$: C, 50.15; H, 5.96; N, 4.87. Found: C, 50.07; H, 5.94; N, 4.79.

Ethyl 2-Ethyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4d) A solution of **7d** (1.80 g, 6.59 mmol) in tetrahydrofuran (THF) (20 ml) was added to a stirred suspension of $LiAlH_4$ (1.05 g, 27.6 mmol) in THF (30 ml) at 0—5 °C. Stirring was continued at the same temperature for 30 min, then water (2 ml) and 5 N NaOH (2 ml) were added successively to the reaction mixture and insoluble material was filtered off. The filtrate was evaporated to dryness, the residue was extracted with AcOEt, and the extract was washed with water and dried (Na_2SO_4). The solvent was distilled off, and the residue was dissolved in benzene (100 ml). *p*-Toluenesulfonic acid hydrate (0.17 g) was added to this solution. The mixture was stirred at room temperature for 30 min and then washed with 4% aqueous $NaHCO_3$ and water, and dried (Na_2SO_4). After removal of the solvent, the residue was chromatographed on silica gel with $CHCl_3$. The eluate was evaporated and the resultant solid was recrystallized from Et_2O–hexane to give 0.99 g (58%) of **4d** as pale yellow crystals. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J=7 Hz), 1.32 (3H, t, J=7 Hz), 2.50 (2H, q), 2.7—3.1 (2H, m), 3.9—4.2 (2H, m), 4.26 (2H, q, J=7 Hz), 6.30 (1H, s).

Ethyl 2-Isopropyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4e) The title compound was prepared from **7e** (1.74 g, 6.06 mmol) using $LiAlH_4$ (0.48 g, 12.6 mmol) in the same manner as described above. Pale yellow crystals (0.93 g, 57%, recrystallized from Et_2O–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.1—1.5 (9H, m), 2.4—3.1 (3H, m), 4.0—4.3 (2H, m), 4.12 (2H, q, J=7 Hz), 6.28 (1H, s).

Method D. 3-Hydroxymethyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4r) A solution of **4j** (2.20 g, 7.31 mmol) in THF (20 ml) was added to a stirred suspension of $LiAlH_4$ (1.55 g, 40.8 mmol) in THF (30 ml) at 0—5 °C. Stirring was continued at room temperature for 1 h, then water (5 ml) and 5 N NaOH (5 ml) were added successively to the reaction mixture and insoluble material was filtered off. The filtrate was evaporated to dryness and the residue was extracted with $CHCl_3$. The extract was washed with water and dried (Na_2SO_4). After removal of the solvent, the residue was chromatographed on silica gel with a mixture of $CHCl_3$ and AcOEt (1 : 1). The eluate was evaporated and the solid was recrystallized from MeOH to give 0.99 g (58%) of **4r** as pale yellow crystals. ^{1}H-NMR ($CDCl_3+D_2O$) δ: 1.28 (3H, t, J=7 Hz), 2.8—3.1 (2H, m), 4.17 (2H, q, J=7 Hz), 4.1—4.4 (2H, m), 4.40 (1H, s), 6.35 (1H, s).

3-Hydroxyethyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4s) The title compound was prepared from **4k** (2.30 g, 7.30 mmol) using $LiAlH_4$ (0.96 g, 25.3 mmol) in the same manner as described above. Pale yellow crystals (0.58 g, 29%, recrystallized from Et_2O–hexane). ^{1}H-NMR ($CDCl_3+D_2O$) δ: 1.34 (3H, t, J=7 Hz), 2.76 (3H, t, J=7 Hz), 2.9—3.1 (2H, m), 3.92 (2H, t, J=7 Hz), 4.1—4.3 (2H, m), 4.29 (2H, q, J=7 Hz), 6.15 (1H, s).

Method E. 2-Methylcarbamoyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4n) A suspension of **4h** (1.12 g, 3.72 mmol) in 40% aqueous $MeNH_2$ (100 ml) was stirred at room temperature for 24 h. Water (100 ml) was added to the mixture. The precipitate was collected and washed with water. The solid was recrystallized from MeOH to give 0.77 g (72%) of **4n** as yellow crystals. ^{1}H-NMR (DMSO-d_6) δ: 1.36 (3H, t, J=7 Hz), 2.6—3.2 (2H, m), 3.19 (3H, s), 4.0—4.6 (4H, m), 7.50 (1H, s). Observed signals were very broad except for a triplet signal at 1.36 (δ).

3-Carbamoyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4o) The title compound was prepared from **4j** (2.70 g, 8.97 mmol) using 28% aqueous NH_3 (100 ml) in the same manner as described above. Yellow crystals (0.95 g, 37%, recrystallized from EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.30 (3H, t, J=7 Hz), 2.8—3.0 (2H, m), 4.19 (2H, q, J=7 Hz), 4.4—4.6 (2H, m), 7.11 (1H, s).

3-Methylcarbamoyl-5,6-dihydrothiazolo[2,3-*c*][1,4]thiazine-8-carboxylate (4p) The title compound was prepared from **4j** (2.23 g, 7.41 mmol) using 40% aqueous $MeNH_2$ (100 ml) in the same manner as described above. Yellow crystals (1.12 g, 53%, recrystallized from EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, t, J=7 Hz), 2.8—3.1 (5H, m), 4.16 (2H, q, J=7 Hz), 4.4—4.6 (2H, m), 6.68 (1H, s).

Pharmacology. Measurement of Galactosamine-Induced Liver Injury Male Sprague-Dawley rats (6 weeks old) were used. They were randomly assigned to groups of 6 animals each. Test compounds were suspended in 0.5% carboxymethylcellulose solution and administered orally at 100 mg/kg and 300 mg/kg. The injection volume was kept at 5 ml/kg and an equal volume of the vehicle was administered to the control group. One hour after the administration of test compounds or vehicle, animals were treated with D-galactosamine at a subcutaneous dose of 800 mg/kg, and starved for 24 h. D-Galactosamine (D-galactosamine·HCl, Sigma) was dissolved in physiological saline and injected at a volume of 5 ml/kg. The blood was then collected from the vena cava under light ether anesthesia. The serum GPT activity was measured using an autoanalyzer system (Impact 400; Gilford). The suppressive effect of each compound against galactosamine-induced liver injury was evaluated in terms of the suppression (%) of the elevation of GPT activity. The suppression (%) was calculated from the following equation.

$$\text{suppression (\%) of elevation of GPT activity} = \{1-([GPT]_T-[GPT]_N)/([GPT]_D-[GPT]_N)\}\times 100$$

where $[GPT]_T$ is the mean GPT activity of the test compound group, $[GPT]_N$ is that of the normal control group, and $[GPT]_D$ is that of the galactosamine-treated control group. Statistical analysis was carried out using Fisher's multiple comparison test for unpaired variates.

Measurement of Monoclonal Antibody(MoAb)-Induced Liver Injury Male Sprague-Dawley rats (6 weeks old) were used. They were randomly assigned to groups of 5 animals each. Test compounds were suspended in 0.5% carboxymethylcellulose solution and administered intraperitoneally at 30 mg/kg. The injection volume was kept at 5 ml/kg and an equal volume of the vehicle was administered to the control group. One hour after the administration of a test compound or vehicle, animals were treated with Millipore-filtered MoAb ascites[2] at an intravenous dose of 5 ml/kg. At 1 h after administration of MoAb ascites, the blood was collected from the vena cava under light ether anesthesia. The serum GPT activity was measured using an autoanalyzer system (Impact 400; Gilford). The suppressive effect of each compound against MoAb-induced liver injury was evaluated in terms of the suppression (%) of the elevation of GPT activity. The suppression (%) was calculated from the equation described above. The statistical analysis was carried out using Student's t test.

References and Notes

1) R. Lesch, W. Reutter, *Verhandl. Deut. Ges. Inn. Med.*, **75**, 358 (1969).
2) T. Ikeda, Y. Kurebayashi, *Hepatology*, **13**, 1152 (1991); Y. Kurebayashi, K. Sato, T. Ikeda, K. Katami, H. Ogawa, Y. Osada, *Igakunoayumi*, **146**, 179 (1988).
3) G. Satzinger, *Arzneim.-Forsch., Drug Res.*, **27**, 463 (1977); M. Herrmann, E. Birkenmayer, V. Ganser, W. Heldt, W. Steinbrecher, *ibid.*, **27**, 467 (1977).
4) S. Hirooka, T. Tanaka, M. Nokata, M. Katoh, T. Sugimoto, *Iyakuhin Kenkyu*, **13**, 1046 (1982).
5) For example; Y. Saiga, I. Iijima, A. Ishida, T. Miyagishima, K. Homma, T. Ohishi, M. Matsumoto, Y. Matsuoka, *Chem. Pharm. Bull.*, **35**, 3284 (1987).
6) R. N. Henrie, II, R. A. Lazaus, S. J. Benkovie, *J. Med. Chem.*, **26**, 559 (1983).
7) T. K. Clausen, S. Scheibye, O. S. Lawesson, "Organic Synthesis," Coll. Vol. VII, ed. by J. P. Freeman, John Wiley and Sons Inc., New York, 1990, pp. 372—375.

8) R. H. Wiley, D. C. England, L. C. Behr, "Organic Reactions," Vol. VI, John Wiley and Sons Inc., New York, 1951, pp. 367—409.
9) G. Y. Balon, D. M. Shulman, *Zh. Org. Khim.*, **15**, 2351 (1979).
10) T. Okawara, K. Nakayama, M. Furukawa, *Heterocycles*, **19**, 1571 (1982)
11) R. O. C. Norman, W. J. E. Parr, C. B. Thomas, *J. Chem. Soc., Perkin Trans. 1*, **1976**, 811.
12) A. Dornow, F. Borberg, L. Schürer, *Arch. Pharm.*, **286**, 494 (1953) [*Chem. Abstr.*, **49**, 8302*c* (1955)].
13) G. W. Holton, G. Parker, A. Robertson, *J. Chem. Soc.*, **1949**, 2049.
14) 1,1-Dichloro-2-propanol was prepared by reduction of 1,1-dicholoropropanone (commercially available) using sodium borohydride. The physical data of the product was coincided with those reported by B. M. Goldschmidt *et al.* (B. M. Goldschmidt, B. L. V. Duuren and R. C. Goldstein, *Tetrahedron Lett.*, **1979**, 1177.)
15) E. Fisher, H. Sheibler, *Chem. Ber.*, **41**, 2891 (1908).