Synthesis of 3-Substituted 2-Oxo-1,4-thiazines and Evaluation of Their Protective Effect against Endotoxin Shock in Mouse

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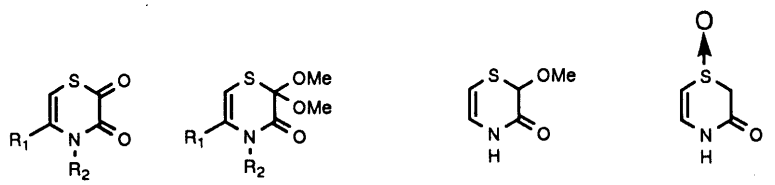
5-Methyl-2,3-dioxo-2*H*,4*H*-1,4-thiazine (7) was obtained by the oxidation of 5-methyl-2*H*-1,4-thiazin-3(4*H*)-one (5) with *m*-chloroperbenzoic acid in MeOH, followed by acid hydrolysis of the resulting 2,2-dimethoxy-1,4-thiazine (6). 3-Chloro-2-oxo-1,4-thiazine (10), which was obtained from 7 by heating with phosphorous oxychloride, reacted with various nucleophiles to give 3-substituted 2-oxo-1,4-thiazines (11a—y). Some of these 2-oxo-1,4-thiazines, 11a—b, e, o and r—s, showed a protective effect against endotoxin shock in D-galactosamine-sensitized mice.

Keywords 2-oxo-1,4-thiazine; 3-substituted 2-oxo-1,4-thiazine; endotoxin shock; D-galactosamine

In a previous paper,[1] we described the synthesis and inotropic activities of pyridyl-1,4-thiazinones. As a continuation of that work, our attention has been focused on the chemical and biological properties of 1,4-thiazines. Recently, Hojo *et al.*[2] reported the synthesis of *N*-alkyl 2,3-dioxo-1,4-thiazine derivatives (**1**) *via* acid hydrolysis of 2,2-dimethoxy-1,4-thiazines (**2**). Bobek[3] reported the synthesis of *N*-unsubstituted 2-methoxy-1,4-thiazine (**3**) *via* a Pummerer-type reaction of 1,4-thiazine 1-oxide (**4**). However, further synthetic or biological studies on these 2-oxo or 2-alkoxy compounds have not been reported. In this paper, we describe the synthesis of *N*-unsubstituted 5-methyl-2,3-dioxo-2*H*,4*H*-1,4-thiazine derivatives (**7**) and their biological activities.

5-Methyl-2*H*-1,4-thiazin-3(4*H*)-one (**5**) was prepared by the method of Rao *et al.*[4] and treated with 2 molar eq of *m*-chloroperbenzoic acid (*m*CPBA) in MeOH, giving 2,2-dimethoxy-5-methyl-2*H*-1,4-thiazin-3(4*H*)-one (**6**) in a good yield. On the other hand, when **5** was treated with 1 equimolar amount of *m*CPBA in acetone, the 1,4-thiazine 1-oxide (**8**) was obtained. When a methanol solution of **8** was allowed to stand at room temperature, the 2-methoxythiazinone (**9**) was obtained. Compound **9** was obtained directly from **5** when MeOH was used as a solvent instead of acetone in the oxidation procedure with *m*CPBA.

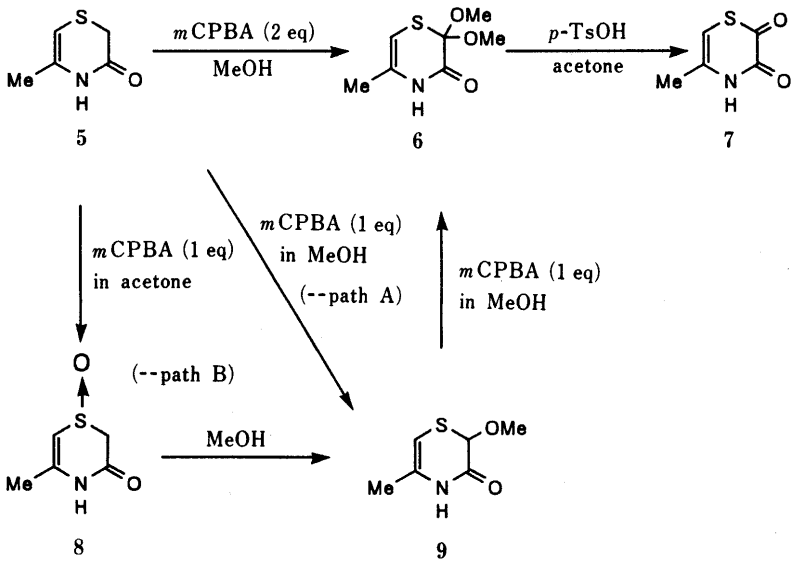
When **9** was treated again with 1 equimolar amount of *m*CPBA in MeOH, the 2,2-dimethoxy-1,4-thiazine (**6**) was obtained. Presumably, the methanolysis may have occurred



1 2 3 4

R_1=H, Me; R_2=Me, iso-Pro

Chart 1



5 6 7 8 9

Chart 2

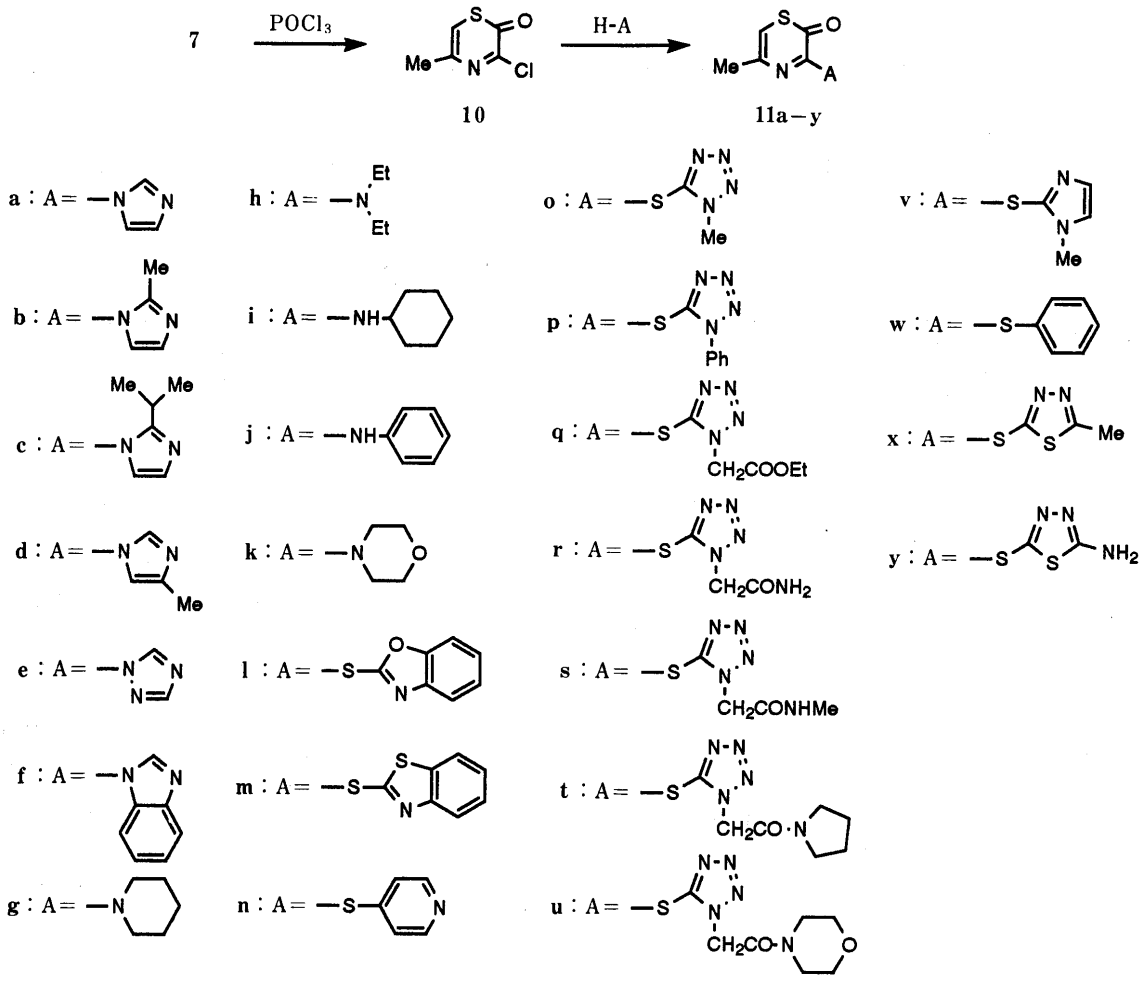


Chart 3

via a sulfoxide intermediate, although we could not isolate 2-methoxy-1,4-thiazine 1-oxide. Acid hydrolysis of **6** proceeded smoothly with *p*-toluenesulfonic acid in an aqueous acetone solution to give the 2,3-dioxo-1,4-thiazine (**7**) in a good yield.

5-Methyl-2,3-dioxo-1,4-thiazine (**7**) was obtained as a yellow crystalline material, which is very soluble in water. The infrared (IR) spectrum of **7** showed overlapping absorptions of two carbonyl groups near 1640 cm^{-1} and its proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed a doublet at δ 2.22 (J=1.0 Hz) due to methyl protons at C-5 and a quartet at δ 5.67 (J=1.0 Hz) due to H-6, both being allylically coupled with the formal doublet. Moreover the elemental analysis is consistent with the proposed structure of **7**.

Chlorination of **7** (at C-3) was achieved by heating with phosphorous oxychloride in the presence of 1 equimolar amount of *N*,*N*-dimethylformamide (DMF) in $(CH_2)_2Cl_2$ at 80 °C, giving 3-chloro-2-oxo-1,4-thiazine (**10**) as an unstable yellow solid. 3-Chloro-2-oxo-1,4-thiazine (**10**) reacted with various aromatic amines or thioles in appropriate solvents to give corresponding 3-substituted 2-oxothiazines (**11a**—**y**). The ^{1}H-NMR spectra of these compounds showed singlets at δ 6.8—7.2 due to a proton at the 6-position of the thiazine. Other spectral data and elemental analyses were consistent with the illustrated structures of **11a**—**y**.

Table I. Effects of 3-Substituted 2-Oxo-1,4-thiazines **11** on Endotoxin Shock in GALN-Sensitized Mice

Compd.[a]	ED_{50} (mg/kg)[b]	Compd.[a]	ED_{50} (mg/kg)[b]
11a	33 (17—64)	**11o**	<25
11b	40 (28—56)	**11p**	>100
11c	>100	**11q**	>100
11e	25 (15—41)	**11r**	18.5 (7.8—23.4) i.p.
11f	58 (36—92)	**11s**	14.8 (9.7—29.8) i.p.
11g	>100	**11t**	>100
11h	>100	**11u**	>100
11i	<50	**11v**	50 (33—77)
11j	>100	**11w**	>100
11k	>100	**11x**	≥50
11l	79 (49—130)	**11y**	>100
11m	73 (49—108)	Dexamethasone	138
11n	≥100		

a) Samples and dexamathasone were administered intraperitoneally (i.p.) 1 h before GALN-endotoxin was injected. *b*) ED_{50} (mg/kg) was calculated by using the Litchfield–Wilcoxon method. Parentheses indicate 95% confidence limits.

Biological Results

Endotoxins (lipopolysaccharides: LPS) derived from the cell walls of gram-negative bacteria have many biological properties such as pyrogenicity, induction of hypotension, activation of complement and so on. Septic shock due to gram-negative bacterial infection is a serious clinical problem, although several treatments are available for this shock syndrome.

We tested compounds (**11a**—**y**) for preventive effect

against mortality in the D-galactosamine (GALN)-sensitized murine endotoxin shock model.[5] The results of this screening are summarized in Table I. Several 3-substituted 2-oxo-1,4-thiazines **11a—b, e, o** and **r—s** showed more potent activity than that of dexamethasone. These compounds will be subjected to further pharmacological investigation, *e.g.* for protective effect against rat endotoxin shock and inhibitory effect on activation of complement.

Experimental

Melting points were determined on a Yamato MP-1 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL FX-270 spectrometer with tetramethylsilane as the reference, and IR spectra were recorded on a Hitachi 260-10 spectrometer. The results of detailed characterization (yields, elemental analyses, IR, and ^{1}H-NMR spectra) of the compounds reported here are summarized in Table II. Thin-layer chromatography (TLC) was performed on TLC plates, Silica gel $60F_{254}$ precoated, layer thickness 0.2 mm (E. Merck) and spots were detected under ultraviolet (UV) irradiation. Column chromatography was done on Wakogel C-200 and the developing solvents are shown in parentheses.

2,2-Dimethoxy-5-methyl-2*H*-1,4-thiazin-3(4*H*)-one (6) *m*CPBA (86.3 g, 0.4 mol) was added portionwise to a solution of 5-methyl-2*H*-1,4-thiazin-3(4*H*)-one (**5**) (25.8 g, 0.2 mol) in MeOH (520 ml) at 0 °C with stirring. Then, the mixture was stirred at room temperature for 20 h. After further

TABLE II. Data for 3-Substituted 2-Oxo-1,4-thiazines **11**

Compd.	mp (°C) (Recrystn. solvent)	IR (KBr) cm^{-1}	^{1}H-NMR ($CDCl_3$)[a]	Analysis (%) Calcd (Found) C	H	N	Formula	Yield (%)
11a	126—127 (EtOAc)	1640, 1570	2.42 (3H, s), 6.97 (1H, s), 7.13 (1H, d, J=1 Hz), 7.94 (1H, d, J=1 Hz), 8.75 (1H, s)	49.73 (49.76	3.65 3.57	21.75 21.70)	$C_8H_7N_3OS$	62
11b	130—132 (Column, EtOAc)	1620, 1570	2.41 (3H, s), 2.56 (3H, s), 6.96 (1H, d, J=1.6 Hz), 7.05 (1H, s), 7.47 (1H, d, J=1.6 Hz)	52.16 (52.16	4.38 4.31	20.27 20.11)	$C_9H_9N_3OS$	39
11c	89.5—92 (Column, EtOAc)	2980, 1640, 1620, 1580	1.33 (3H, s), 1.35 (3H, s), 2.42 (3H, s), 3.28 (1H, m), 7.01 (1H, d, J=1.6 Hz), 7.07 (1H, s), 7.32 (1H, d, J=1.6 Hz)	56.15 (56.15	5.57 5.63	17.86 18.11)	$C_{11}H_{13}N_3OS$	33
11d	158—160 (ab. EtOH)	1620, 1590	2.27 (3H, s), 2.40 (3H, s), 6.90 (1H, s), 7.63 (1H, s), 8.66 (1H, s)	52.16 (52.09	4.38 4.35	20.27 20.10)	$C_9H_9N_3OS$	66
11e	91.5—93 (ab. EtOH)	1640, 1570	2.54 (3H, s), 7.14 (1H, s), 8.18 (1H, s), 9.38 (1H, s)	43.29 (43.36	3.11 3.08	28.85 28.77)	$C_7H_6N_4OS$	37
11f	200—201 (EtOAc)	1610, 1560	2.51 (3H, s), 6.94 (1H, s), 7.38 (2H, m), 7.82 (1H, m), 8.32 (1H, m), 9.17 (1H, s)	59.24 (59.38	3.73 3.67	17.27 17.31)	$C_{12}H_9N_3OS$	35
11g	83—83.5 (EtOH)	3100, 3030, 1600	1.50—1.80 (6H, m), 2.50 (3H, s), 3.40—4.60 (4H, m), 7.13 (1H, s)	57.12 (56.84	6.71 6.74	13.32 13.00)	$C_{10}H_{14}N_2OS$	81
11h	Oil (Column, $CHCl_3$)	3100, 1620	1.23 (6H, t, J=7 Hz), 2.49 (3H, s), 3.10—4.40 (4H, m), 7.14 (1H, s)	54.51 (54.08	7.12 7.09	14.13 13.64)	$C_9H_{14}N_2OS$	37
11i	Oil (Column, $CHCl_3$)	3300, 3100, 1660	1.10—2.20 (11H, m), 2.47 (3H, s), 3.97 (1H, br), 7.13 (1H, s)	58.90 (59.00	7.19 7.16	12.49 11.97)	$C_{11}H_{16}N_2OS$	100
11j	Oil (Column, $CHCl_3$)	3380, 3300, 1680, 1600	2.53 (3H, s), 7.20—8.00 (6H, s+m), 9.20 (1H, br)	60.53 (61.17	4.62 4.58	12.83 12.84)	$C_{11}H_{10}N_2OS$	98
11k	87—88 (Column, $CHCl_3$)	1600, 1550	2.50 (3H, s), 3.50—4.80 (8H, m), 7.17 (1H, s)	50.93 (50.29	5.70 5.53	13.20 12.69)	$C_9H_{12}N_2O_2S$	42
11l	148—150 (EtOH)	1600, 1560	2.09 (3H, s), 6.77 (1H, s), 7.41 (2H, m), 7.58 (1H, m), 7.81 (1H, m)	52.16 (52.22	2.92 2.80	10.14 10.10)	$C_{12}H_8N_2O_2S_2$	74
11m	183—185 (DMF)	1610, 1560	2.48 (3H, s), 6.82 (1H, s), 7.39—7.53 (2H, m), 7.93 (1H, m), 8.03 (1H, m)	49.29 (49.16	2.76 2.58	9.58 9.56)	$C_{12}H_8N_2OS_3$	55
11n	117—120 (EtOAc–hexane)	1630, 1560	2.18 (3H, s), 6.72 (1H, s), 7.50 (2H, d, J=6.3 Hz), 8.65 (2H, d, J=6.3 Hz)	50.83 (50.62	3.41 3.32	11.85 12.03)	$C_{10}H_8N_2OS_2$	46
11o	185—191 (dec.) (DMF)	1610, 1560	2.13 (3H, s), 4.08 (3H, s), 6.82 (1H, s)	34.85 (34.86	2.92 2.82	29.03 29.08)	$C_7H_7N_5OS_2$	77
11p	192—197 (dec.) (EtOAc)	3050, 1600	2.50 (3H, s), 7.37 (1H, s), 7.57—7.72 (5H, m)	47.51 (47.50	2.99 2.87	23.09 22.90)	$C_{12}H_9N_5OS_2$	54
11q	134—135 (EtOAc–hexane)	3000, 1750, 1620	1.28 (3H, t, J=7 Hz), 2.14 (3H, s), 4.27 (2H, q, J=7 Hz), 5.14 (2H, s), 6.82 (1H, s)	38.33 (38.40	3.54 3.50	22.35 22.13)	$C_{10}H_{11}N_5O_3S_2$	42
11r	192—197 (dec.)	3380, 1680, 1610	2.50 (3H, s), 5.16 (2H, s), 7.43 (1H, s), 7.51 (1H, br s), 7.73 (1H, br s)	33.80 (33.77	2.84 2.70	29.56 29.21)	$C_8H_8N_6O_2S_2$	39
11s	170—183 (dec.) (EtOAc)	3320, 1660, 1610	2.05 (3H, s), 2.56 (3H, d, J=4.6 Hz), 5.15 (2H, s), 8.25 (1H, br)	36.23 (36.40	3.38 3.30	28.17 27.83)	$C_9H_{10}N_6O_2S_2$	21
11t	175—181 (dec.) (EtOH)	1650, 1610	1.87—2.10 (4H, m), 2.13 (3H, s), 3.47—3.53 (4H, m), 5.14 (2H, s), 6.78 (1H, s)	42.59 (42.49	4.17 4.14	24.83 24.53)	$C_{12}H_{14}N_6O_2S_2$	30
11u	180—186 (dec.) (EtOH)	1650, 1610	2.13 (3H, s), 3.47—3.55 (2H, m), 3.58—3.65 (2H, m), 3.70—3.80 (4H, m), 5.24 (2H, s), 6.80 (1H, s)	40.67 (40.65	3.98 3.99	23.71 23.32)	$C_{12}H_{14}N_6O_3S_2$	28
11v	136—139 (ab. EtOH)	1620, 1560	2.13 (3H, s), 3.68 (3H, s), 6.68 (1H, s), 7.19 (1H, d, J=1 Hz), 7.24 (1H, d, J=1 Hz)	45.17 (44.82	3.79 3.78	17.56 17.38)	$C_9H_9N_3OS_2$	60
11w	97—98 (ab. EtOH)	1620, 1550	2.11 (3H, s), 6.60 (1H, s), 7.42 (3H, m), 7.52 (2H, m)	56.15 (55.86	3.86 3.69	5.95 6.05)	$C_{11}H_9NOS_2$	34
11x	175—180 (dec.) (Column, $CHCl_3$–MeOH)	1620, 1560	2.43 (3H, s), 2.85 (3H, s), 6.85 (1H, s)	37.34 (37.29	2.74 2.62	16.33 16.33)	$C_8H_7N_3OS_3$	36
11y	174—176 (dec.) (EtOH)	3430, 1600	2.27 (3H, s), 7.43 (1H, s), 7.57 (2H, br s)	32.55 (32.59	2.34 2.18	21.69 21.50)	$C_7H_6N_4OS_3$	35

a) Chemical shifts are given with proton numbers, absorption patterns and coupling constants in parentheses.

addition of crystalline anhydrous K_2CO_3 (5.3 g, 0.4 mol), the mixture was heated at 40—50 °C for 0.5 h and the resulting precipitates were filtered off. The filtrate was evaporated to dryness *in vacuo* and the residue was dissolved in $CHCl_3$–water (1 : 1, v/v). The $CHCl_3$ layer was collected, washed with a saturated NaCl solution, dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was allowed to stand at 0 °C overnight and the resulting solid was washed with petroleum ether to give **6** (24.2 g, 63.8%) as pale yellow prisms, mp 93—95 °C. IR (KBr) cm^{-1}: 3200, 3080, 1680. ^{1}H-NMR ($CDCl_3$) δ: 2.00 (3H, s), 3.56 (3H, s), 5.33 (1H, s), 8.86 (1H, br).

2-Methoxy-5-methyl-2*H*-1,4-thiazin-3(4*H*)-one (9) *m*CPBA (4.2 g, 19.4 mmol) was added portionwise to a mixture of **5** (2.5 g, 19.4 mmol) and MeOH (70 ml) at 0 °C with stirring. The mixture was stirred at room temperature for 2 d, then evaporated to dryness *in vacuo*, and the residue was dissolved in $CHCl_3$, washed with saturated $NaHCO_3$ solution and successively with water, and then dried over anhydrous $MgSO_4$. The solvent was evaporated off *in vacuo* and the residual solid was washed with ether to give **9** (1.9 g, 82.6%) as a pale yellow powder, mp 157—160 °C. IR (KBr) cm^{-1}: 3200, 3100, 1680, 1640. ^{1}H-NMR ($CDCl_3$) δ: 2.00 (3H, s), 3.44 (3H, s), 4.79 (1H, s), 5.12 (1H, s), 8.53 (1H, br).

5-Methyl-2*H*-1,4-thiazin-3(4*H*)-one 1-Sulfoxide (8) *m*CPBA (2.2 g, 10 mmol) was added to a solution of **5** (1.3 g, 10 mmol) in acetone (30 ml) at 0 °C with stirring. After stirring of the mixture for 15 min, the resulting precipitates were collected and washed with acetone to give **8** (1.2 g, 80%) as a pale yellow powder, mp > 110 °C (dec.). IR (KBr) cm^{-1}: 3200, 3100, 1700, 1640, 1610, 1015. *Anal.* Calcd for $C_5H_7NO_2S$: C, 41.36; H, 4.86; N, 9.65. Found: C, 41.25; H, 4.85; N, 9.27.

Reaction of 8 with MeOH (Path B in Chart 2) A solution of **8** (100 mg, 0.69 mmol) in MeOH (3 ml) was stirred at room temperature for 2.5 h. The solvent was evaporated off *in vacuo* and the residue was washed with ether to give **9** (110 mg, quantitative), which was identical with the sample obtained from path A.

5-Methyl-2,3-dioxo-2*H*,4*H*-1,4-thiazine (7) *p*-Toluenesulfonic acid monohydrate (5.7 g, 0.03 mmol) was added to a stirred solution of **6** (28.4 g, 0.15 mol) in 5% aqueous acetone (50 ml) and the mixture was heated at 50 °C for 0.5 h. After cooling, the resulting precipitates were collected and washed with acetone–water (20 : 1, v/v) and successively with ether. Recrystallization from EtOH gave **7** (16.8 g, 78%) as yellow prisms, mp 195—197 °C. IR (KBr) cm^{-1}: 3200, 3050, 1640, 1610. ^{1}H-NMR ($CDCl_3$) δ: 2.22 (3H, d, J = 1.0 Hz), 5.67 (1H, q, J = 1.0 Hz), 9.90 (1H, br).

3-(1-Imidazolyl)-5-methyl-2-oxo-2*H*-1,4-thiazine (11a) and Its Analogs (11b—y) As a typical example, the preparation of **11a** is described. Phosphorous oxychloride (1.96 ml, 21 mmol) was added to a mixture of **7** (2 g, 14 mmol), DMF (1.4 ml, 18 mmol) and dichloroethane (30 ml) with stirring. Then, the mixture was stirred at 80 °C for 0.5 h. After cooling, the mixture was poured into ice-water and extracted several times with ethyl acetate. The combined extracts were washed with saturated $NaHCO_3$ solution and dried over anhydrous $MgSO_4$. The solvent was evaporated off *in vacuo* and the residue was purified by chromatography on a short column of silica gel (ethyl acetate) to give 3-chloro-5-methyl-2-oxo-2*H*-1,4-thiazine (**10**) as yellow needles. This product was used in the next reaction without further purification. IR (KBr) cm^{-1}: 1640.

Imidazole (1.4 g, 21 mmol) was added portionwise to a mixture of **10**, CH_2Cl_2 (30 ml) and triethylamine (2.9 ml, 21 mmol) with stirring. The mixture was stirred at room temperature for 48 h, then poured into saturated NaCl solution and extracted with $CHCl_3$. The extract was dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The residue was recrystallized from ethyl acetate to give the imidazole derivative **11a**.

The melting points, yields, spectral data and elemental analyses of **11b**—**y** are given in Table II.

Animals Male ICR mice, weighing 25—28 g, were purchased from Charles River, Co., Ltd. and kept for 7 d under specific-pathogen-free conditions in our laboratory before use. Animals were fasted overnight before experiments.

Endotoxin Shock Model with GALN-Induced Fulminant Hepatitis Both 400 mg/kg of GALN (Wako) and 15 μg/kg of endotoxin (*E. coli* 0111 : B4 lipopolysaccharide : Sigma) were given simultaneously to animals *via* the tail vein. The number of survivors was recorded after 1 d.

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