Synthesis and Pharmacological Activities of Novel Cyclic Disulfide and Cyclic Sulfide Derivatives as Hepatoprotective Agents

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In order to search for anti-hepatitis drugs, we synthesized a series of eight- and nine-membered cyclic disulfides (1) and six- and seven-membered cyclic sulfides (2) and evaluated them for ability to reduce mortality in the model of acute hepatic failure induced by *Propionibacterium acnes*-lipopolysaccharide in mice. Compounds 1 were synthesized by oxidative cyclization of the corresponding dithiol derivatives (3) with diethyl bromomalonate or iodine. Compounds 2 were prepared from the methyl esters of 1 by desulfurization with tris(diethylamino)phosphine followed by deprotection. Compounds 1 were generally found to be more active than compounds 2. Compound 1b (SA3443) was found to exhibit potent protective activity. The synthesis and structure–activity relationships are discussed.

Keywords anti-hepatitis drug; cyclic disulfide; cyclic sulfide; *Propionibacterium acnes*-lipopolysaccharide; structure–activity relationship; SA3443

Immune reaction is considered to play an important role in the pathogenesis of certain human liver injuries such as acute viral hepatitis, primary biliary cirrhosis and alcohol- or drug-induced hepatitis.[1] Therefore, immunologically induced liver injury models should be used in screening for new anti-hepatitis drugs. Recently, *Propionibacterium acnes*-lipopolysaccharide (*P. acnes*-LPS)-induced hepatitis has been used as an immunologically based liver injury model.[2] We therefore adopted this model to search for new hepatoprotective agents.

In a series of studies aimed at the development of new drugs, we have synthesized various mercaptoacylamino acids[3] and have developed an anti-rheumatic drug, bucillamine, [*N*-(2-mercapto-2-methylpropionyl)-L-cysteine].[4] Bucillamine affects the immune systems.[5] Moreover, cyclic disulfide compound SA981, one of the metabolites of bucillamine, has been reported to have immunomodulatory effects.[6] It was also reported that some disulfides, such as thioctic acid and pantethine, showed inhibitory effects against liver injury, as well as immunomodulatory actions.[7]

In order to search for new anti-hepatitis drugs, structural modifications of SA981, as the lead compound, were made and eight- and nine-membered cyclic disulfides (**1**) and six- and seven-membered cyclic sulfides (**2**) were designed (Chart 1). We evaluated these compounds for protective activity in the acute hepatic failure model induced by *P. acnes*-LPS in mice.

In this paper we describe the chemical synthesis and structure–activity relationships of **1** and **2**.

Chemistry

The desired compounds (listed in Table I) were synthesized by the routes shown in Charts 2—5.

Methods for the Synthesis of the Cyclic Disulfides 1 The cyclic disulfides (**1**) were synthesized by the procedures shown in Chart 2. Compounds **1** were prepared from **3** having two thiol groups by oxidative cyclization with diethyl bromomalonate (method A) or iodine (method B). Kato *et al.* reported that SA981 was obtained by oxidative cyclization of bucillamine with diethyl bromomalonate in the presence of triethylamine.[8] We applied this reaction to the synthesis of **1c—e**, **1g** and **1h** and obtained these compounds in 18.1—70.1% yield (method A). Compounds **1a**, **1b** and **1f** were synthesized by oxidative cyclization with iodine.[9] In this reaction, dilution of the reaction

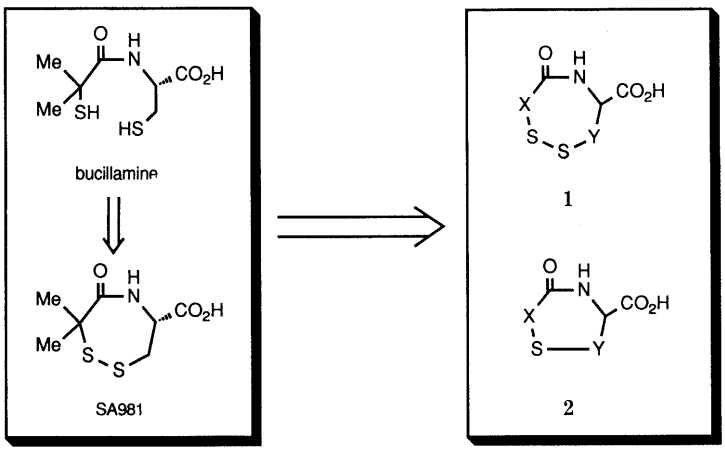


Chart 1

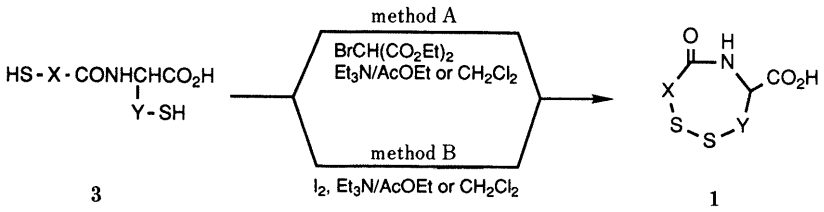
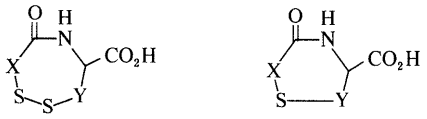


Chart 2

TABLE I. Physicochemical Data for the Cyclic Disulfides (**1**) and Cyclic Sulfides (**2**)



1 **2**

Compd. No.	X[a)]	Y	Confign.	Method	Yield (%)	mp (°C)	Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	$[\alpha]^{25}$ (°) (c=1.0, MeOH)
1a	$(CH_2)_2$	CH_2	*R*	B	24.9	135—137 (dec.)	MeOH	$C_6H_9NO_3S_2 \cdot 1/2MeOH$	34.96 (34.80	4.97 4.99	6.27 6.21)	−67.6
1b (SA3443)	$Me_2CC^*H_2$	CH_2	*R*	B	63.2	163—164	EtOH–H_2O	$C_8H_{13}NO_3S_2$	40.83 (40.70	5.57 5.58	5.95 5.95)	−112.2
1c	$Me_2CC^*H_2$	CH_2	*S*	A	53.8	159.5—161.5	AcOEt	$C_8H_{13}NO_3S_2$	40.83 (40.62	5.57 5.78	5.95 5.96)	+110.2
1d	$CH_2C^*Me_2$	CH_2	*R*	A	23.4	250—252 (dec.)	MeOH–AcOEt	$C_8H_{13}NO_3S_2$	40.83 (40.81	5.57 5.53	5.95 5.88)	−49.7
1e	$Me_2CC^*H_2$	Me_2C	*S*	A	18.1	214.5—216.5	AcOEt	$C_{10}H_{17}NO_3S_2$	45.60 (45.56	6.51 6.70	5.32 5.23)	+403.8
1f	$Et_2CC^*H_2$	CH_2	*R*	B	57.8	127—128.5	AcOEt–hexane	$C_{10}H_{17}NO_3S_2$	45.60 (45.63	6.51 6.69	5.32 5.28)	−90.3
1g	Me_2C	$(CH_2)_2$	*RS*	A	70.1	210.5—212	EtOH–H_2O	$C_8H_{13}NO_3S_2$	40.83 (40.70	5.57 5.61	5.95 5.93)	—
1h	$Me_2CC^*H_2$	$(CH_2)_2$	*RS*	A	30.5	206—208	AcOEt	$C_9H_{15}NO_3S_2$	43.35 (43.47	6.06 6.17	5.62 5.45)	—
2a	Me_2C	CH_2	*RS*	—	68.2	268.5—270	EtOH–hexane	$C_7H_{11}NO_3S$	44.43 (44.66	5.86 6.02	7.40 7.28)	—
2b	$Me_2CC^*H_2$	CH_2	*RS*	—	81.8	218—221 (dec.)	EtOH–hexane	$C_8H_{13}NO_3S$	47.27 (47.12	6.45 6.54	6.89 6.84)	—
2c	Me_2C	$(CH_2)_2$	*RS*	—	58.6	266—268 (dec.)	EtOH	$C_8H_{13}NO_3S$	47.27 (47.22	6.45 6.74	6.89 6.58)	—

a) * indicates the binding site with S.

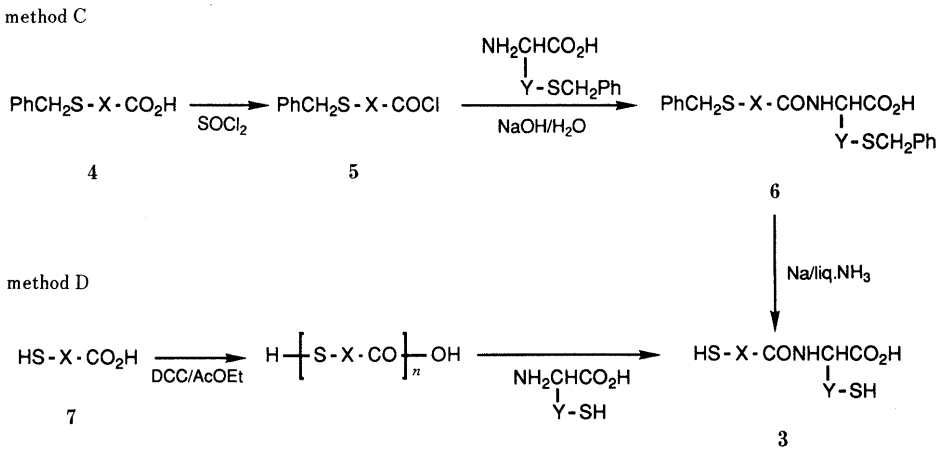


Chart 3

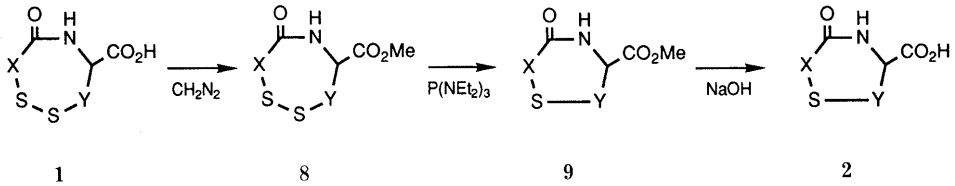
solution improved the yield by reducing the intermolecular reaction. Thus, a solution of a dithiol compound (**3**) and iodine are added separately and slowly to a solution of triethylamine in AcOEt or CH_2Cl_2. We obtained these compounds in 24.9—63.2% yield. The physicochemical data of **1** are summarized in Table I.

Compounds **3** were synthesized according to methods C and D shown in Chart 3. In method C, *S*-benzyl-*N*-benzylthioacylmercaptoamino acids (**6**) were prepared by the reaction of *S*-benzylalkanoyl chloride (**5**) with *S*-benzylmercaptoamino acids in the presence of a base such as sodium hydroxide. Compounds **6** were deprotected with metallic sodium in liquid ammonia to give mercaptoacylmercaptoamino acids (**3**). In method D, mercaptoalkanoic acids (**7**) provided the corresponding polythioesters by reaction with *N*,*N*′-dicyclohexylcarbodiimide (DCC). The resulting polythioesters were converted to **3** by coupling with mercaptoamino acids.

Methods for the Synthesis of the Cyclic Sulfides 2 Compounds **2** were synthesized as shown in Chart 4. Harpp and Gleason have reported demonosulfurization of disulfide with tris(diethylamino)phosphine.[10] The symmetrical disulfides are desulfurized in high yield to afford the corresponding thioethers. However, few unsymmetrical disulfides were examined. We were able to apply this reaction to prepare our unsymmetrical cyclic disulfides.

Compounds **1b**, **1g** and SA981 were esterified with diazomethane to afford **8a—c** in 91.0—93.1% yield. Compounds **8a—c** were then desulfurized with tris(diethylamino)phosphine at 52—54 °C in tetrahydrofuran (THF) for 2 h to give the cyclic sulfides **9a—c** in 13.8—55.1% yield. Since we could not obtain the optically active products in the case of the desulfurization of **8a** and **8b**, compounds **9a** and **9b** were racemized in this reaction. Hydrolysis of **9a—c** with methanolic aqueous NaOH gave the desired compounds **2a—c** in 58.6—81.8% yield. The physicochemical properties of **2** are summarized in Table I.

Methods for the Synthesis of Benzylthioalkanoic Acids 4 and Mercaptoalkanoic Acids 7 Compounds **4** and **7** were synthesized as shown in Chart 5. 2-Benzylthiomethyl-2-ethylbutanoic acid (**4a**) was prepared as follows. The methylester (**11**) was obtained by the reaction of methyl 2-ethylbutyrate (**10**) with benzylthiomethyl chloride, which was prepared from benzylmercaptan and *s*-trioxane[11] in the presence of lithium diisopropylamide. Hydrolysis of **11** with alkaline solution gave the desired carboxylic acid (**4a**). 3-Benzylthio-2,2-dimethylpropanoic acid (**4b**) was obtained by the reaction of 3-chloro-2,2-dimethylpropanoic acid (**12**) with benzylmercaptan. 3-Mercapto-2,2-dimethylpropanoic acid (**7a**) was obtained by the reaction of **12** with thiourea followed by alkaline hydrolysis.

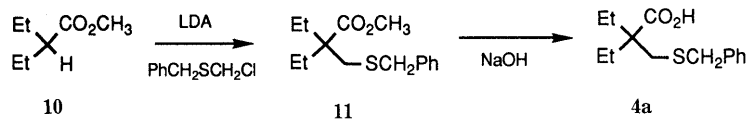


1 8 9 2

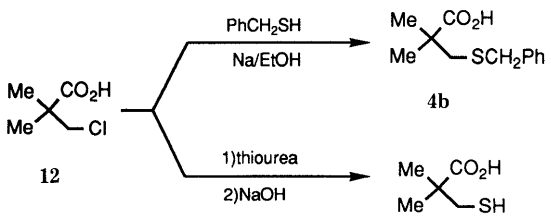
1	8	9	2	X	Y
SA981	a	a	a	Me_2C	CH_2
b	b	b	b	$Me_2CC^*H_2$	CH_2
g	c	c	c	Me_2C	$(CH_2)_2$

* binding site with S

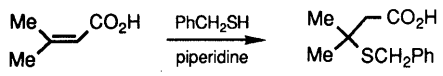
Chart 4



10 11 4a



4b 12 7a



4c

Chart 5

3-Benzylthio-3-methylbutanoic acid (**4c**) was prepared by addition of benzylmercaptan to 3,3-dimethylacrylic acid.[12]

Biological Results

Compounds **1** and **2** were tested for the ability to reduce the mortality of mice with acute hepatic failure induced by *P. acnes*-LPS as described in the experimental section, and their activities are listed in Table II.

As shown in Table II, when LPS (25 μg/mouse) was injected into BALB/C mice previously treated with heat-killed *P. acnes*, 80% of the animals died of acute hepatic failure within 48 h after injection. Compound **1b** (SA3443) greatly reduced the mortality of the mice at the dose of 100 mg/kg, *p.o.*

Structure–activity relationships are summarized in Chart 6.

a) A carboxyl group with (*R*)-configuration (**1b**) increased the activity more effectively than that with (*S*)-configuration (**1c**).

b) As regards the length and the steric factors of the linking group between the sulfur atom and the asymmetric carbon atom, a methylene group (**1b**) resulted in higher activity than a dimethyl substituted group (**1e**) or an ethylene group (**1g, h**).

c) Dimethylation of the carbon atom at the β-position to the amidocarbonyl group led to a decrease of the activity.

d) Conversion of the substituent at the α-position to the amidocarbonyl group from a dimethyl (**1b**) to a hydrogen (**1a**) or a diethyl (**1f**) reduced the activity.

e) Compounds **1** were generally more active than compounds **2**.

In conclusion, we designed and synthesized the cyclic disulfides **1** and cyclic sulfides **2** from SA981 as the lead compound and evaluated their ability to reduce mortality in the acute hepatic failure model induced by *P. acnes*-LPS. SA3443 [**1b**, (4*R*)-hexahydro-7,7-dimethyl-6-oxo-1,2,5-dithiazocine-4-carboxylic acid] was found to possess a hepatoprotective activity. This compound also has protective effects against other kinds of immunological hepatic injury[2b] and chemically (CCl_4, DL-ethionine or acetaminophene) induced acute hepatic injury.[13]

TABLE II. Effects of the Cyclic Disulfides (**1**) and Cyclic Sulfides (**2**) on the Mortality of Mice with Acute Hepatic Failure Induced by *P. acnes*-LPS

Compd.[a] No.	Mortality (no. dead/total) (%) 0—48 h	
Control	4/5	80
SA981	3/5	60
1a	3/5	60
1b (SA3443)	1/5	20
1c	4/5	80
1d	4/5	80
1e	5/5	100
1f	5/5	100
1g	2/5	40
1h	4/5	80
2a	4/5	80
2b	2/5	40
2c	5/5	100

a) Test compounds were given orally at the dose of 100 mg/kg 1 h before LPS injection.

Experimental

Chemistry All melting points were determined in open glass capillaries with a Yamato MP-21 melting point apparatus and are uncorrected. Elemental analyses were performed by a Yanagimoto MT-3 CHN corder element analyzer. IR spectra were recorded on a JASCO A302 infrared spectrophotometer. Specific rotations were measured at the sodium D line with a JASCO DIP-140 digital polarimeter. ^{1}H-NMR spectra were measured on a JEOL GSX 400 spectrometer. Chemical shifts are expressed as δ (ppm) values with tetramethylsilane as an internal standard and coupling constants are given in hertz (Hz); s, d, t, q, m, dd, dt, ddd and br refer to singlet, doublet, triplet, quartet, multiplet, double doublet, double triplet, double double doublet and broad, respectively. Organic extracts were dried over anhydrous $MgSO_4$, then the solvent was evaporated off under reduced pressure. Silica gel BW-300 (Fuji Devison) was used for column chromatography.

Typical Procedures for the Preparation of Cyclic Disulfides 1 (4*S*)-Hexahydro-7,7-dimethyl-6-oxo-1,2,5-dithiazocine-4-carboxylic Acid (**1c**) Method A: A solution of *N*-(3-mercapto-2,2-dimethylpropionyl)-D-cysteine (**3b**, 15.0 g, 63.2 mmol) in CH_2Cl_2 (1 l) was added to a stirred solution of diethyl bromomalonate (16.6 g, 69.5 mmol) and triethylamine (13.4 g, 133 mmol) in CH_2Cl_2 (6 l) at −12—−17 °C over a period of 6 h. The mixture was stirred at the same temperature for 30 min and at room temperature for 30 min, acidified with aqueous 1 N HCl and washed with brine. The organic layer was concentrated and the residue was column-chromatographed on silica gel ($CHCl_3$: MeOH = 100 : 1—50 : 1). The product was recrystallized from AcOEt to afford **1c** (8.0 g, 53.8%), mp 159.5—161.5 °C.

Compounds **1d**, **1e**, **1g** and **1h** were prepared in a similar manner to that described for **1c**.

(4*R*)-Hexahydro-7,7-dimethyl-6-oxo-1,2,5-dithiazocine-4-carboxylic Acid (**1b**) Method B: A solution of *N*-(3-mercapto-2,2-dimethylpropionyl)-L-cysteine (**3a**, 31.0 g, 130 mmol) in AcOEt (440 ml) and a solution of iodine (33.0 g, 130 mmol) in AcOEt (440 ml) were added dropwise to a stirred solution of triethylamine (29.0 g, 290 mmol) in AcOEt (430 ml) at −10—+5 °C for 3 h. The mixture was stirred at the same temperature for 1 h and the precipitates of triethylamine hydroiodide were removed. The filtrate was washed successively with aqueous 1 N HCl, aqueous 1% $NaHSO_3$, H_2O and brine and concentrated. The precipitated crystals were collected and recrystallized from EtOH–H_2O (1 : 5) to afford **1b** (19.4 g, 63.2%), mp 163—164 °C.

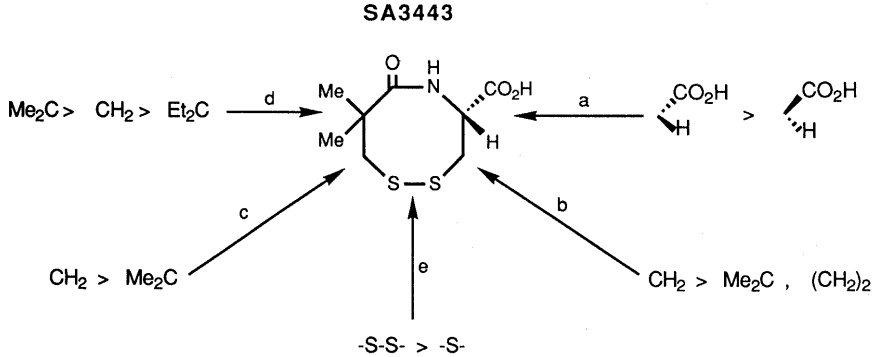
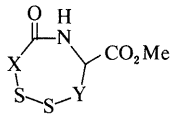


Chart 6

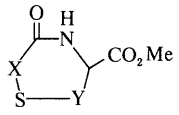
TABLE III. ^{1}H-NMR and IR Spectral Data for the Cyclic Disulfides (**1**)

Compd. No.	IR (KBr, cm^{-1})	NMR (DMSO-d_6)
1a	1757, 1624, 1490	2.12—2.28, 2.35—2.46, 2.72—3.12, 3.26—3.35 (total 6H, each m, $CH_2 \times 3$), 3.63—3.70, 4.00—4.22, 4.60—4.90 (total 1H, each m, CH), 7.06—7.52, 7.98—8.10, 8.00—8.43 (total 1H, each m, NH)
1b (SA3443)	1730, 1627, 1507	0.95, 1.06, 1.26, 1.28 (total 6H, each s, $CH_3 \times 2$), 2.95—3.52 (4H, m, $CH_2 \times 2$), 3.61—3.67, 4.67—4.86 (total 1H, each m, CH), 7.56, 8.42 (total 1H, each d, J=9.3, 6.3, NH), 12.4—13.2 (1H, br, CO_2H)
1c	1729, 1625, 1505	0.95, 1.07, 1.26, 1.28 (total 6H, each s, $CH_3 \times 2$), 2.95—3.51 (4H, m, $CH_2 \times 2$), 3.61—3.66, 4.70—4.82 (total 1H, each m, CH), 7.55, 8.40 (total 1H, each d, J=9.3, 6.8, NH), 12.2—13.0 (1H, br, CO_2H)
1d	1720, 1608, 1419	1.29, 1.31, 1.36, 1.39, 1.46 (total 6H, each s, $CH_3 \times 2$), 2.05, 2.09, 2.14 (total 1H, each d, J=13.2, 11.2, 13.2, $Me_2C\underline{H}_a$), 2.70, 2.76 (total 1H, each t, dd, J=12.7 (t), 14.6 (d), 9.8 (d), $CHC\underline{H}_a$), 2.96, 2.99, 3.08 (total 1H, each d, J=12.2, 13.2, 13.2, $Me_2C\underline{H}_b$), 3.16—3.25 (1H, m, $CHC\underline{H}_b$), 4.65, 4.67, 4.77 (total 1H, each dt, dt, t, J=3.4 (d), 11.7 (t), 3.4 (d), 11.7 (t), 9.8 (t), CH), 6.96, 7.34, 7.79 (total 1H, each d, J=8.8, 11.2, 11.2, NH)
1e	1733, 1644, 1529	1.12 (3H, s, CH_3), 1.31 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.48 (3H, s, CH_3), 2.85 (1H, d, J=13.9, CH_a), 2.97 (1H, d, J=13.9, CH_b), 4.70 (1H, d, J=10.7, CH), 6.92 (1H, d, J=10.7, NH), 13.10—13.20 (1H, br, CO_2H)
1f	1713, 1642, 1518	0.67—0.79 (6H, m, $C\underline{H}_3CH_2 \times 2$), 1.30—1.84 (4H, m, $CH_3C\underline{H}_2 \times 2$), 3.02—3.51 (4H, m, $CH_2S \times 2$), 3.59—3.64, 4.78 (total 1H, each m, s, CH), 7.48, 8.37 (total 1H, each d, J=9.3, 6.8, NH), 12.2—13.2 (1H, br, CO_2H)
1g	1701, 1653, 1520	1.33, 1.37 (total 3H, each s, CH_3), 1.54, 1.57 (total 3H, each s, CH_3), 2.18—2.42, 2.53—2.66, 3.08—3.16, 3.26—3.46 (total 4H, each m, $CH_2 \times 2$), 3.60—3.69, 4.36—4.46, 4.64—4.76 (total 1H, each m, CH), 7.02—7.12, 7.69, 8.37 (total 1H, each m, d, d, J=10.3, 6.8, NH), 12.35—13.00 (1H, br, CO_2H)
1h	1716, 1624, 1538	1.03 (3H, s, CH_3), 1.19 (3H, s, CH_3), 1.93 (1H, dt, J=15.1 (d), 11.2 (t), $CHCH_2C\underline{H}_a$), 2.30 (1H, ddd, J=15.1, 7.3, 4.9, $CHCH_2C\underline{H}_b$), 2.35—2.49 (1H, m, $CHC\underline{H}_a$), 2.91 (1H, d, J=14.7, $Me_2CC\underline{H}_a$), 3.24 (1H, d, J=14.7, $Me_2CC\underline{H}_b$), 3.15—3.28 (1H, m, $CHC\underline{H}_b$), 4.65—4.77 (1H, m, CH), 8.00—8.15 (1H, m, NH)

TABLE IV. Physicochemical Data for **8** and **9**



8



9

Compd. No.	X[a)]	Y	Confign.	Yield (%)	mp (°C)	Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	$[\alpha]^{25}$ (°) (c=1.0, MeOH)
8a	Me_2C	CH_2	*R*	91.7	53—54	AcOEt–hexane	$C_8H_{13}NO_3S_2$	40.83 (40.76	5.57 5.52	5.95 5.92)	+158.4
8b	$Me_2CC^*H_2$	CH_2	*R*	93.1	87—89	AcOEt–hexane	$C_9H_{15}NO_3S_2$	43.35 (43.22	6.06 6.18	5.62 5.60)	−128.9
8c	Me_2C	$(CH_2)_2$	*RS*	91.0	210—212	Benzene–hexane	$C_9H_{15}NO_3S_2$	43.35 (43.21	6.06 6.19	5.62 5.59)	—
9a	Me_2C	CH_2	*RS*	13.8	142—144	AcOEt–hexane	$C_8H_{13}NO_3S$	47.27 (47.40	6.45 6.51	6.89 6.86)	—
9b	$Me_2CC^*H_2$	CH_2	*RS*	23.6	89—90.5	AcOEt–hexane	$C_9H_{15}NO_3S$	49.75 (49.61	6.96 7.05	6.45 6.29)	—
9c	Me_2C	$(CH_2)_2$	*RS*	55.1	172—174	AcOEt	$C_9H_{15}NO_3S$	49.75 (49.59	6.96 7.09	6.45 6.24)	—

a) See footnote *a* in Table I.

Compounds **1a** and **1f** were prepared in a similar manner to that described for **1b**.

The ^{1}H-NMR and IR spectral data of **1** are summarized in Table III.

Typical Procedures for the Preparation of Cyclic Sulfides 2 Methyl (6*RS*)-Hexahydro-3,3-dimethyl-4-oxo-1,2,5-dithiazocine-6-carboxylate (**8c**): A ether solution of diazomethane (1.1 l) was added dropwise to a stirred solution of (6*RS*)-hexahydro-3,3-dimethyl-4-oxo-1,2,5-dithiazocine-6-carboxylic acid (**1g**, 28.0 g, 119 mmol) and 41.7% HCl/AcOEt (10 ml) in MeOH (700 ml) at 10—20 °C. The mixture was stirred at the same temperature for 5 min. After cooling, the mixture was treated with acetic acid (10 ml) and concentrated. The precipitated crystals were recrystallized from benzene–hexane (1 : 2) to afford **8c** (27.0 g, 91.0%), mp 210—212 °C. IR (KBr): 1728, 1644, 1520 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 1.32, 1.37 (total 3H, each s, CH_3), 1.54, 1.57 (total 3H, each s, CH_3), 2.19—2.28, 2.30—2.42, 2.51—2.68, 3.10—3.17, 3.59—3.65 (total 4H, each m, $CH_2 \times 2$), 3.59, 3.65 (total 3H, each s, OCH_3), 3.71—3.80, 4.78—4.88 (total 1H, each m, CH), 7.84, 8.47 (total 1H, each d, J=9.3, 6.8, NH).

Methyl (5*RS*)-Hexahydro-2,2-dimethyl-3-oxo-1,4-thiazepine-5-carboxylate (**9c**): A solution of tris(diethylamino)phosphine (59.5 g, 241 mmol) in THF (140 ml) was added dropwise to a stirred solution of **8c** (12.0 g, 48 mmol) in THF (1.2 l) at 52—54 °C. The mixture was stirred at the same temperature for 2 h and concentrated. The crude crystals were column-chromatographed on silica gel (AcOEt) and recrystallized from AcOEt to afford **9c** (5.76 g, 55.1%), mp 172—174 °C. IR (KBr): 1738, 1647, 1509 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 1.38, 1.41, 1.49 (total 6H, each s, $CH_3 \times 2$), 1.75—1.85, 1.86—1.97, 2.03—2.17, 2.38—2.61 (total 4H, each m, $CH_2 \times 2$), 3.59, 3.60 (total 3H, each s, OCH_3), 4.16, 4.30 (total 1H, each ddd, J=11.2, 7.8, 2.9 and J=11.2, 7.3, 3.4, CH), 8.07, 8.31 (total 1H, each d, J=7.3, 7.8, NH).

(5*RS*)-Hexahydro-2,2-dimethyl-3-oxo-1,4-thiazepine-5-carboxylic Acid (**2c**): An aqueous 1 N NaOH solution (20 ml) was added to a stirred solution of **9c** (2.17 g, 10 mmol) in MeOH (220 ml) at 0—5 °C. The mixture was stirred with cooling for 4 h, diluted with H_2O, acidified with aqueous 6 N HCl and extracted with AcOEt. The extract was washed with brine and evaporated. The residue was column-chromatographed on silica gel ($CHCl_3$: MeOH = 20 : 1) and recrystallized from EtOH–hexane (1 : 2) to afford **2c** (1.3 g, 58.6%), mp 266—268 °C (dec.).

Table V. ^{1}H-NMR and IR Spectral Data for the Cyclic Sulfides (**2**)

Compd. No.	IR (KBr, cm^{-1})	NMR (DMSO-d_6)
2a	1728, 1711, 1679, 1647, 1520	1.43 (3H, s, CH_3), 1.44 (3H, s, CH_3), 2.84 (1H, dd, J=13.7, 4.4, $CHC\underline{H}_a$), 3.08 (1H, dd, J=13.7, 7.3, $CHC\underline{H}_b$), 4.33 (1H, dt, J=4.4 (d), 7.3 (t), CH), 8.06 (1H, d, J=7.3, NH), 12.97 (1H, s, CO_2H)
2b	1725, 1557, 1480	1.15 (3H, s, CH_3), 1.21 (3H, s, CH_3), 2.51 (1H, d, J=15.1, $Me_2CC\underline{H}_a$), 2.67 (1H, dd, J=12.7, 10.7, $CHC\underline{H}_a$), 2.83 (1H, d, J=15.1, $Me_2CC\underline{H}_b$), 3.10 (1H, dd, J=12.7, 3.4, $CHC\underline{H}_b$), 4.57 (1H, ddd, J=10.7, 6.4, 3.4, CH), 6.53 (1H, d, J=6.4, NH), 13.4—13.7 (1H, br, CO_2H)
2c	1743, 1614, 1517	1.38, 1.40, 1.49 (total 6H, each s, $CH_3 \times 2$), 1.78—2.16, 2.36—2.60 (total 4H, each m, $CH_2 \times 2$), 4.07—4.14, 4.21—4.30 (total 1H, each m, CH), 7.85, 8.12 (total 1H, each d, J=7.3, 7.8, NH), 12.57 (1H, s, CO_2H)

Table VI. Physicochemical Data for the Dithiol Derivatives **3**

HS–X–CONHCHCO$_2$H
　　　　　　|
　　　　　Y–SH

3

Compd. No.	X[a)]	Y	Confign.	Method	Yield (%)	mp (°C)	Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	$[\alpha]^{25}$ (°) (c=1.0, $CHCl_3$)
3a	$Me_2CC^*H_2$	CH_2	*R*	D	63.5	83—84.5	AcOEt–cyclohexane	$C_8H_{15}NO_3S_2$	40.48 (40.38	6.37 6.57	5.90 5.86)	+57.2
3b	$Me_2CC^*H_2$	CH_2	*S*	C	71.2	83.5—85	AcOEt–cyclohexane	$C_8H_{15}NO_3S_2$	40.48 (40.47	6.37 6.37	5.90 5.94)	−57.1
3c	$CH_2C^*Me_2$	CH_2	*R*	C	71.2	109.5—112	Benzene	$C_8H_{15}NO_3S_2$	40.48 (40.55	6.37 6.65	5.90 5.89)	+44.8
3d	$Me_2CC^*H_2$	Me_2C	*S*	D	44.2	115.5—117	AcOEt–hexane	$C_{10}H_{19}NO_3S_2$	45.26 (45.12	7.22 7.44	5.28 5.06)	+18.3 (MeOH)
3e	$Et_2CC^*H_2$	CH_2	*R*	C	73.5	83.5—84.5	AcOEt–hexane	$C_{10}H_{19}NO_3S_2$	45.26 (45.21	7.22 7.58	5.28 5.18)	+50.6 (20 °C)
3f	Me_2C	$(CH_2)_2$	*RS*	D	57.8	99.5—101	(iso-Pr)$_2$O	$C_8H_{15}NO_3S_2$	40.48 (40.34	6.37 6.53	5.90 5.92)	—
3g	$Me_2CC^*H_2$	$(CH_2)_2$	*RS*	D	54.4	105—106	AcOEt–hexane	$C_9H_{17}NO_3S_2$	43.00 (42.92	6.82 7.00	5.57 5.49)	—

a) See footnote *a* in Table I.

Compounds **8a**, **8b**, **9a**, **9b**, **2a** and **2b** were prepared in a similar manner to that described for **8c**, **9c** and **2c**. Their physicochemical properties are summarized in Tables I and IV. The ^{1}H-NMR and IR spectral data of **2** are summarized in Table V.

Typical Procedures for the Preparation of Dithiol Compounds 3 *N*-(3-Mercapto-2,2-dimethylpropionyl)-D-cysteine (**3b**) Method C: 1) Thionyl chloride (21.1 ml, 290 mmol) and DMF (2 drops) were added to 3-benzylthio-2,2-dimethylpropanoic acid (**4b**, 50.0 g, 223 mmol). The mixture was stirred at room temperature for 2 h, concentrated and distilled under reduced pressure to afford 3-benzylthio-2,2-dimethylpropionyl chloride (**5b**, 53.0 g, 98.0%). bp 124—127 °C (0.5 mmHg).

2) A solution of **5b** (53.0 g, 218 mmol) in Et_2O (25 ml) was added dropwise to a stirred solution of *S*-benzyl-D-cysteine (41.9 g, 198 mmol) in aqueous 2 N NaOH (248 ml, 496 mmol) and Et_2O (20 ml) with ice-cooling. The mixture was stirred at the same temperature for 20 min and at room temperature for 2 h. The mixture was acidified with aqueous 6 N HCl and extracted with AcOEt. The extract was washed with brine and concentrated. The residue was column-chromatographed on silica gel (benzene : AcOEt = 9 : 1—8 : 2) to afford *S*-benzyl-*N*-(3-benzylthio-2,2-dimethylpropionyl)-D-cysteine (**6b**, 65 g, 78.5%) as an oil.

3) Metallic sodium (17.2 g, 750 mmol) was added in small portions to a stirred solution of **6b** (63.0 g, 150 mmol) in liquid NH_3 (800 ml) under an N_2 atmosphere at −78 °C. The mixture was stirred at the same temperature for 45 min. Ammonium chloride was added and ammonia was removed by distillation. The residue was dissolved in H_2O and washed with AcOEt. The aqueous layer was acidified with aqueous 6 N HCl and extracted with AcOEt. The extract was washed with brine and concentrated. The residue was column-chromatographed on silica gel (benzene : AcOEt : AcOH = 100 : 5 : 1—100 : 10 : 1) to afford **3b** (25.5 g, 71.2%). The crystals were recrystallized from AcOEt–cyclohexane (1 : 3), mp 83.5—85 °C. IR (KBr): 1725, 1624, 1522 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.32 (3H, s, CH_3), 1.33 (3H, s, CH_3), 1.51 (1H, t, J=9.0, SH), 1.56 (1H, t, J=9.0, SH), 2.66 (1H, dd, J=13.7, 9.0, $Me_2CC\underline{H}_a$), 2.76 (1H, dd, J=13.7, 9.0, $Me_2CC\underline{H}_b$), 3.08 (1H, ddd, J=14.2, 9.0, 3.9, $CHC\underline{H}_a$), 3.12 (1H, ddd, J=14.2, 9.0, 3.9, $CHC\underline{H}_b$), 4.88 (1H, dt, J=7.3, 3.9, CH), 6.79 (1H, d, J=7.3, NH), 8.5—8.9 (1H, br, CO_2H).

Compounds **3c** and **3e** were prepared in a similar manner to that described for **3b**.

N-(2-Mercapto-2-methylpropionyl)-DL-homocysteine (**3f**) Method D: A solution of *N*,*N'*-dicyclohexylcarbodiimide (69.3 g, 336 mmol) in AcOEt (200 ml) was added dropwise to a stirred solution of 2-mercapto-2-methylpropanoic acid (40.4 g, 336 mmol) in AcOEt (200 ml) under an N_2 atmosphere at 5—10 °C. The mixture was stirred at room temperature for 40 min. The precipitated *N*,*N'*-dicyclohexylurea was collected and washed with AcOEt. The filtrate was combined with the washings and concentrated to 200 ml. The AcOEt solution of polythioester was added to a stirred solution of DL-homocysteine (37.9 g, 280 mmol) and K_2CO_3 (77.4 g, 560 mmol) in H_2O (400 ml) and *N*,*N*-dimethylformamide (DMF) (100 ml). The mixture was stirred overnight at room temperature, diluted with H_2O and washed with AcOEt. The aqueous layer was acidified with aqueous 6 N HCl and extracted with AcOEt. The extract was washed with brine and concentrated. The residue was column-chromatographed on silica gel (benzen : AcOEt : AcOH = 100 : 5 : 1—100 : 10 : 1) and recrystallized from (iso-Pr)$_2$O to afford **3f** (38.5 g, 57.8%), mp 99.5—101 °C. IR (KBr): 1712, 1599, 1524 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.62 (6H, s, $CH_3 \times 2$), 1.62 (1H, t, J=8.3, $CH_2S\underline{H}$), 2.05—2.14 (1H, m, $CHC\underline{H}_a$), 2.21—2.30 (1H, m, $CHC\underline{H}_b$), 2.31 (1H, s, $Me_2CS\underline{H}$), 4.72 (1H, dt, J=7.8, 5.4, CH), 7.55 (1H, d, J=7.8, NH), 8.8—9.2 (1H, br, CO_2H).

Compounds **3a**, **3d** and **3g** were prepared in a similar manner to that described for **3f**. The physicochemical data of **3** are summarized in Table VI.

Procedures for the Preparation of Benzylthioalkanoic Acids 4 and Mercaptoalkanoic Acids 7 2-Benzylthiomethyl-2-ethylbutanoic Acid (**4a**): 1) A solution of methyl 2-ethylbutyrate (**10**, 21.2 g, 163 mmol) in THF

TABLE VII. Physicochemical Data for **4**, **5**, **6** and **7**

$PhCH_2S$–X–CO_2H (**4**) $PhCH_2S$–X–COCl (**5**) $PhCH_2S$–X–$CONHCHCO_2H$ (**6** Y: CH_2) HS–X–CO_2H (**7**)

Compd. No.	X[a]	Confign.	Yield (%)	mp or bp (°C)	IR (film, cm^{-1})	NMR ($CDCl_3$)	$[\alpha]^{25}$ (°) (c=1.0, MeOH)
4a	$Et_2CC^*H_2$	—	98.2	Oil	1693	0.77 (6H, t, J=7.6, $CH_3CH_2\times2$), 1.66 (4H, q, J=7.6, $CH_3CH_2\times2$), 2.72 (2H, s, CH_2SCH_2Ph), 3.71 (2H, s, CH_2SCH_2Ph), 7.21—7.34 (5H, m, Ar-H), 9.8—12.2 (1H, br, CO_2H)	—
4b	$Me_2CC^*H_2$	—	80.4	47—49.0	1684 (KBr)	1.25 (6H, s, $CH_3\times2$), 2.67 (2H, s, CH_2SCH_2Ph), 3.74 (2H, s, CH_2SCH_2Ph), 7.21—7.33 (5H, m, Ar-H), 9.9—12.2 (1H, br, CO_2H)	—
4c	$CH_2C^*Me_2$	—	81.0	140—150 (0.7—1.0 mmHg)	1703	1.49 (6H, s, $CH_3\times2$), 2.64 (2H, s, CH_2CO_2H), 3.78 (2H, s, $PhCH_2$), 6.92—7.57 (5H, m, Ar-H), 10.8—11.1 (1H, br, CO_2H)	—
5a	$Et_2CC^*H_2$	—	Quant.	Oil	1794	—	—
5b	$Me_2CC^*H_2$	—	98.0	124—127 (0.5 mmHg)	1794	—	—
5c	$CH_2C^*Me_2$	—	77.5	120—124 (1.5 mmHg)	1799	—	—
6a	$Et_2CC^*H_2$	*R*	93.8	Oil	1732, 1621, 1512	0.76 (3H, t, J=7.3, CH_3CH_2), 0.77 (3H, t, J=7.3, CH_3CH_2), 1.55—1.72 (4H, m, $CH_3CH_2\times2$), 2.68 (1H, d, J=12.7, Et_2CCH_a), 2.73 (1H, d, J=12.7, Et_2CCH_b), 2.89 (1H, dd, J=14.2, 6.6, $CHCH_a$), 2.95 (1H, dd, J=14.2, 5.4, $CHCH_b$), 3.70 (2H, s, $PhCH_2$), 3.72, 3.73 (total 2H, each s, $PhCH_2$), 4.60—5.40 (1H, br, CO_2H), 4.65—4.70 (1H, m, CH), 6.71 (1H, d, J=6.3, NH)	+33.0
6b	$Me_2CC^*H_2$	*S*	78.5	Oil	1729, 1620, 1513	1.24 (3H, s, CH_3), 1.25 (3H, s, CH_3), 2.64 (1H, d, J=12.7, Me_2CCH_a), 2.70 (1H, d, J=12.7, Me_2CCH_b), 2.92 (1H, dd, J=14.2, 5.6, $CHCH_a$), 2.97 (1H, dd, J=14.2, 5.6, $CHCH_b$), 3.71 (2H, s, $PhCH_2$), 3.71 (1H, d, J=12.5, $PhCH_a$), 3.74 (1H, d, J=12.5, $PhCH_b$), 4.70 (1H, ddd, J=6.8, 5.6, 5.6, CH), 6.75 (1H, d, J=6.8, NH), 7.20—7.30 (1H, m, Ar-H), 6.80—7.50 (1H, br, CO_2H)	−38.1
6c	$CH_2C^*Me_2$	*R*	96.0	Oil	1729, 1618, 1523	1.39 (6H, s, $CH_3\times2$), 2.44 (2H, s, CH_2CO), 2.85 (2H, d, J=5.5, CH_2CH), 3.67 (2H, s, $PhCH_2$), 3.74 (2H, s, $PhCH_2$), 4.70—4.95 (1H, m, CH), 6.97—7.54 (11H, m, Ar-H and NH), 10.31 (1H, s, CO_2H)	−47.0 (20 °C)
7a	$Me_2CC^*H_2$	—	77.2	86—90 (2 mmHg)	1696	1.30 (6H, s, $CH_3\times3$), 1.49 (1H, t, J=8.8, SH), 2.71 (2H, d, J=8.8, CH_2), 10.0—12.5 (1H, br, CO_2H)	—

a) See footnote *a* in Table I.

(40 ml) was added to lithium diisopropylamide [prepared *in situ* from *n*-BuLi (1.6 M solution in hexane, 122 ml) and iso-Pr_2NH (19.7 g, 195 mmol) in THF (120 ml) at −78 °C under an N_2 atmosphere] with stirring. Hexamethylphosphoramide (29.2 g, 163 mmol) was added to the reaction mixture at the same temperature. The mixture was stirred at −78 °C for 30 min and at room temperature for 75 min. The mixture was treated with H_2O, concentrated and extracted with AcOEt. The extract was washed with brine and evaporated. The crude residue was column-chromatographed on silica gel (hexane : AcOEt = 50 : 1) to afford methyl 2-benzylthiomethyl-2-ethylbutyrate (**11**, 27.6 g, 79.8%) as an oil. IR (film): 1729 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 0.71 (6H, t, J=7.3, $CH_3CH_2\times2$), 1.64 (4H, q, J=7.3, $CH_3CH_2\times2$), 2.72 (2H, s, $PhCH_2SCH_2$), 3.65 (3H, s, OCH_3), 3.69 (2H, s, $PhCH_2$), 7.22—7.33 (5H, m, Ar-H).

2) A solution of **11** (42 g, 158 mmol) in EtOH (200 ml) was added to a stirred solution of KOH (62.4 g, 946 mmol) in H_2O (200 ml) and the mixture was refluxed for 15.5 h. After removal of the solvent, the residue was acidified with aqueous 6 N HCl and extracted with Et_2O. The extract was washed with brine and evaporated to afford **4a** (39.1 g, 98.2%) as an oil.

3-Benzylthio-2,2-dimethylpropanoic Acid (**4b**): A solution of benzylmercaptan (43 ml, 366 mmol) in EtOH (50 ml) was added to a stirred solution of sodium ethoxide in EtOH [prepared *in situ* from sodium metal (16.8 g, 732 mmol) and EtOH (300 ml)]. 3-Chloro-2,2-dimethylpropanoic acid (**12**, 50 g, 366 mmol) was added dropwise to the reaction mixture at 10—20 °C. The mixture was stirred at room temperature for 80 min and refluxed for 10 min. After cooling, the reaction mixture was diluted with H_2O. After removal of the solvent, the residue was dissolved in H_2O, acidified with aqueous 6 N HCl and extracted with Et_2O. The extract was washed with brine and evaporated. The resulting crystals were collected by filtration to afford **4b** (66.0 g, 80.4%), mp 47.0—49.0 °C.

3-Benzylthio-3-methylbutanoic Acid (**4c**): A mixture of benzylmercaptan (58.7 ml, 500 mmol), 3,3-dimethylacrylic acid (50.0 g, 500 mmol), and piperidine (90 ml) was refluxed for 22 h. The mixture was acidified with aqueous 6 N HCl and extracted with Et_2O. The Et_2O layer was then extracted with aqueous 10% Na_2CO_3. The extract was acidified with aqueous 6 N HCl and extracted with Et_2O. The organic layer was washed with brine and evaporated. The residual oil was distilled under reduced pressure to afford **4c** (90.8 g, 81.0%), bp 140—150 °C (0.7—1.0 mmHg).

3-Mercapto-2,2-dimethylpropanoic Acid (**7a**): A mixture of **12** (100 g, 732 mmol) and thiourea (57 g, 747 mmol) was stirred at 180—200 °C for 2.5 h. After cooling, a solution of NaOH (73.2 g, 1.83 mol) in H_2O (400 ml) was added to the reaction mixture and the whole was refluxed for 2.5 h. After cooling, the mixture was acidified with aqueous 6 N HCl and extracted with AcOEt. The extract was washed with brine and evaporated. The residual oil was distilled under reduced pressure to afford **7a** (74.8 g, 77.2%), bp 86—90 °C (2 mmHg).

The physicochemical properties of **4**—**7** are summarized in Table VII.

Pharmacological Method Male BALB/C mice, weighing about 20 g, were used. *P. acnes* and LPS were obtained from Wellcome Biotechnology Ltd. (Beckenham, England) and Difco (Detroit, Michigan, U.S.A.), respectively. Test compounds were suspended in a 1% methylcellulose solution.

Acute hepatic failure was produced according to the method of Ferluga and Allison.[2a] In brief, heat-killed *P. acnes* (1.0 mg) was injected i.v. into each BALB/C mouse through a tail vein. Seven days later, LPS (25 μg) was injected i.v. and acute hepatic failure was thereby induced. Test compounds were orally administered at the dose of 100 mg/kg 1 h before LPS injection, and the mortality rate was estimated. The mice were starved for 24 h before LPS injection.

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