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Potent and Subtype-Selective CCK-B/gastrin Receptor Antagonists: 2,4-Dioxo-1,5-benzodiazepines with a Plane of Symmetry

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Abstract—A series of CCK-B/gastrin receptor antagonists, 2,4-dioxo-1,5-benzodiazepine derivatives with a plane of symmetry, were designed, synthesized, and evaluated for antagonistic activity. Structure–activity relationship studies revealed that carbonylmethyl groups at both *N*-1 and *N*-5 positions and hydrophilic groups, such as the carboxyl group on the benzene ring attached to the ureido group at the C-3 position, brought about potent affinity and subtype selectivity for CCK-B/gastrin receptors. Several compounds showed excellent in vivo inhibition of gastric acid secretion induced by pentagastrin in anesthetized rats. © 1997 Elsevier Science Ltd.

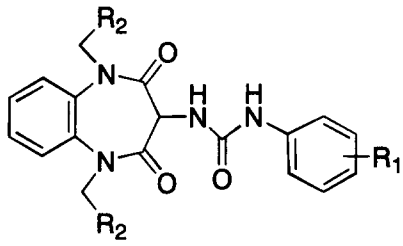
Introduction

Cholecystokinin (CCK) and gastrin belong to the polypeptide hormone family and are found in both the central nervous system (CNS) and gastrointestinal tissue. Several biologically active forms of CCK exist as the C-terminal 58, 33, 8 and 4 peptides. CCK-8 is the predominant form in the CNS. Gastrin acts as a physiological mediator of gastric acid secretion stimulated by meals, and a growth factor for mucosal cells including gastrin-dependent histamine-secretory enterochromaphin-like cells (ECL cells). Both CCK and gastrin are closely related and have an identical C-terminal pentapeptide sequence (Gly-Trp-Met-Asp-Phe-NH_2). The C-terminal tetrapeptide (CCK-4) appeared to be the minimum sequence required for activity on the gastrin receptor.[1]

The receptors for CCK and gastrin have been classified as subtypes CCK-A and CCK-B/gastrin. The CCK-A receptor subtype predominates in peripheral target organs and mediates the control of gall bladder function and digestive enzyme secretion. The CCK-B/gastrin receptor is widely distributed in the CNS and mucosal cells, and is responsible for neurotransmission or neuromodulation, and for gastrin-stimulated acid secretion in the stomach during feeding.[1] Merck scientists discovered the first nonpeptide CCK receptor antagonist, asperlicin, based on a screening program for their fermentation products.[2] Chemical modification using a benzodiazepine pharmacophore produced a potent selective CCK-A receptor antagonist, MK-329, and a CCK-B/gastrin receptor antagonist, L-365,260.[3] Since then, compounds of various structures have been designed and prepared to improve the potency and subtype selectivity.[4]

Many 1,4-benzodiazepine analogues have been synthesized, and recently 1,5-benzodiazepine compounds have been reported to have potent CCK-A receptor antagonistic activity.[5] In general, 2,4-dioxo-1,5-benzodiazepines exist in two enantiomeric forms since 1,5-benzodiazepine compounds have a stereogenic center at C-3 due to the different kinds of substituents at *N*-1 and *N*-5 positions of a 1,5-benzodiazepine skeleton. Regarding its synthetic chemistry, the optical resolution or selective preparation of one enantiomer is still a laborious process. Very recently, the Glaxo Wellcome group reported on symmetrical 2,4-dioxo-1,5-benzodiazepines as CCK-B receptor antagonists.[6] However, these compounds showed a strong decrease in affinity for both CCK-A and B receptors, or a high affinity for CCK-A receptors. In the present study, we examined the synthesis of potent and subtype-selective CCK-B/gastrin receptor antagonists having a plane of symmetry, whose molecules need not be optically resolved or stereoselectively synthesized.

As previously reported,[7] when L-365,260 is superimposed on the folded conformation of *N*-acetyl-CCK-7, Tyr-7 in CCK-7 corresponds to the phenyl-ring at the C-5 position of the 1,4-benzodiazepine ring of L-365,260. As mentioned above, CCK-4 is a minimum amino-acid sequence required for activity on the gastrin receptor, therefore, the substituent at C-5 is thought to be not necessarily an aromatic ring or a ring with π-electrons. Based on these facts, we designed 2,4-dioxo-1,5-benzodiazepine compounds with the same substituents at *N*-1 and *N*-5 (Fig. 1). Furthermore, the chemical modification of substituents at the benzene ring attached to the ureido group at the C-3 position was examined.



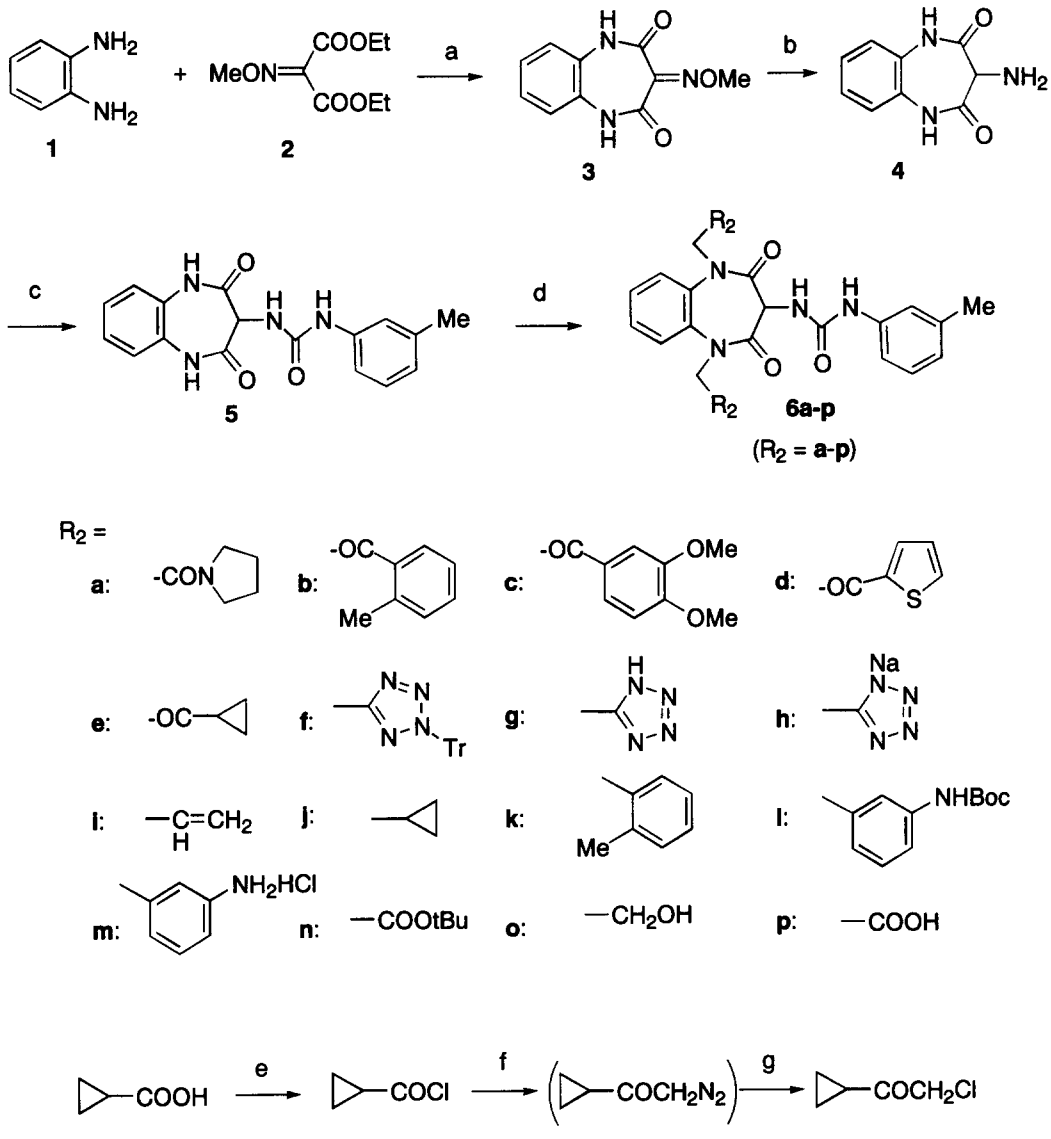
Compound A

Figure 1. Prepared compound A.

Chemistry

1*H*-1,5-Benzodiazepine-2,4-(3H,5H)-dione skeleton was prepared from *o*-phenylenediamine and diethyliminomalonate by heating with sodium methoxide. The methoxime **3** was reduced by catalytic hydrogenation and hydrogenolysis to give an amino compound **4**. First, the substituent at the C-3 position of the 1,5-benzodiazepine skeleton was kept constant with an *m*-tolylureido group, and then chemical modification at the *N*-1 and *N*-5 positions was carried out. Compound **4** was treated with *m*-tolylisocyanate to furnish *m*-tolylureido compound **5**, which was then *N*-alkylated with various kinds of alkyl halide in the presence of K_2CO_3 and KI to give compounds **6a–p**, as depicted in Scheme 1.

From assay results (Table 1) of the affinity for CCK-B/gastrin of prepared compounds, four substituents, such as cyclopropylcarbonylmethyl, pyrrolidinecarbonylmethyl, cyclopropyl, and thienyl-2-ylcarbonylmethyl groups, were chosen as substituents R_2 at *N*-1 and *N*-5 positions. Chemical modification was then carried out



Reagent: a) NaOMe; b) H_2, Pd-C; c) *m*-Me-C_6H_4-NCO; d) R_2CH_2X, K_2CO_3, KI; e) $SOCl_2$; f) CH_2N_2;g) conc.HCl.

Scheme 1.

Table 1. Receptor antagonistic effects and inhibitory effects on gastric acid secretion

Inhibition Compd	Receptors (IC_{50}, nM)			Gastric acid ED_{50} (mg kg^{-1}) (id)
	Gastrin[a]	CCK-B[a]	CCK-A[a]	
6a	7	54	860	>0.30[c]
6b	4	11	29	0.30
6c	>1000[b]	>1000[b]	>10000[b]	
6d	3	17	120	>0.30[c]
6e	5	18	380	0.15
6f	>1000[b]	>1000[b]	460	
6g	>1000[b]	>1000[b]	>10000[b]	
6h	>1000[b]	>1000[b]	>10000[b]	
6i	230	480	2400	
6j	12	23	860	>0.30[c]
6k	32	8	23	
6l	>1000[b]	>1000[b]	13000	
6m	18	21	760	>0.30[c]
6n	2	2	100	>0.10[c]
6o	>1000[b]	>1000[b]	11000	

[a]Binding results are the means of two to four independent determinations.
[b]Full IC_{50} not obtained.
[c]Full ED_{50} not obtained.

at the benzene ring attached to the ureido group at the C-3 position. The synthesis is shown in Scheme 2. After the protection of the amino group of **4** as a BocNH group, *N*-alkylation was achieved with alkyl halide corresponding to these four substituents. Deprotection of the Boc group gave **10a–d**. Alternatively, *N*-alkylation of compound **3** was done with cyclopropylcarbonylmethyl chloride, followed by reduction of the methoxime group with catalytic hydrogenation and hydrogenolysis to give the amino compound **10a**. 3-Amino derivatives **10a–d** were then treated with various isocyanates to give the desired ureido derivatives **11–14** depicted in Scheme 2.

Biology

In vitro receptor-binding assays were used to measure the affinity of compounds for the CCK-B receptor of mouse cortical membranes and the CCK-A receptor of mouse pancreas, respectively. The affinity at the gastrin receptor was measured using guinea pig gastrin glands. IC_{50} values were obtained for the half-maximal inhibition of binding of [propionyl-^{3}H]-CCK-8 (sulfonated) to CCK receptors and of binding human [^{125}I]-gastrin I to the gastrin receptor.[8] In vivo inhibition of gastric acid secretion was measured in anesthetized rats following the administration of pentagastrin with a test compound dosed intraduodenally.[9]

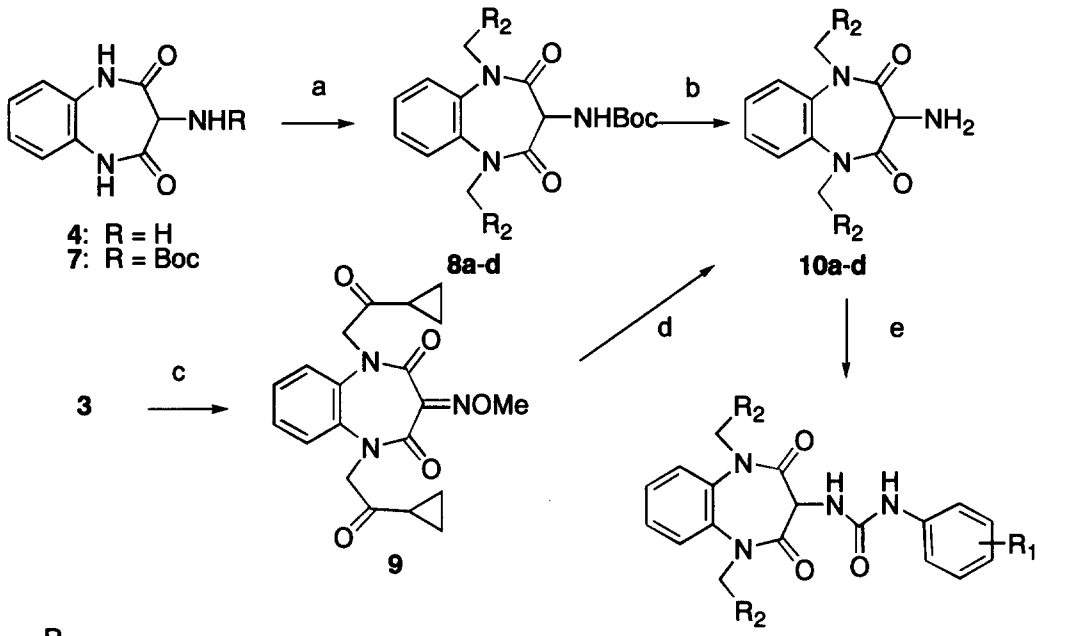
Structure–Activity Relationships and Discussion

First, substituent R_1 of compound A was kept constant with a methyl group since L-365,260, the representative lead compound, had the same methyl group at the corresponding position and showed a potent affinity for the CCK-B receptor. Then, substituents containing amide, carbonyl, tetrazolyl, vinyl, cyclopropyl, aromatic, hydroxyl, ester, and carboxyl groups were introduced to both *N*-1 and *N*-5 positions of the 1,5-benzodiazepine skeleton. The results of binding assays for gastrin, CCK-B, and CCK-A are shown in Table 1. Compounds with sterically bulky substituents, **6c**, **f** and **l**, did not have affinities for the three receptors. In addition, compounds with hydrophilic groups, **6g**, **h** and **o**, did not show the affinities, while **6m** with the *m*-ammonium benzyl group did. The introduction of a carbonyl group into the substituents, **6a**, **b**, **d**, **e** and **n**, increased the gastrin receptor affinity to a nanomolar order, but compounds with vinyl, cyclopropyl, and aromatic groups, **6i–k**, did not show high potency.

Several of the compounds **6** with potent in vitro activity were intraduodenally administrated to examine the inhibition of gastric acid secretion induced by pentagastrin in anesthetized rats. The degrees of inhibition (ED_{50}) are shown in Table 1. Compound **6e**, having a cyclopropylcarbonylmethyl group, showed in vivo efficacy, ED_{50} = 0.15 mg kg^{-1}.

Then, substituents at *N*-1 and *N*-5 positions were kept constant with cyclopropylcarbonylmethyl groups, and chemical modification was carried out at the benzene ring attached to the ureido group at the C-3 position. Furthermore, several compounds with other *N*-1, *N*-5 substituents, such as pyrrolidinecarbonylmethyl, cyclopropylmethyl, and thienylcarbonylmethyl groups, were prepared in case of representative compounds (Table 2).

In our previous study on 2-(*N*-(*tert*-butoxycarbonylmethyl)-*N*-(*N'*-(*m*-carboxyphenyl)ureidomethylcarbonyl)-amino)benzophenone, **S-0509**,[10] we reported that the introduction of a carboxyl group to the benzene ring at the corresponding position increased the activity by one order of magnitude, increased the selectivity of the receptor affinity of gastrin to CCK-A, and further led to



Scheme 2.

poor blood–brain permeability, which increased the selectivity of gastrin from the CCK-B antagonist. Thus, we focused on the mimics of carboxyl or hydrophilic groups.

Compounds with *para*-substitution, **11xiv**, **xv** and **xvi**, decreased affinity for the three receptors. In contrast, all the prepared *meta*-substituted derivatives showed potent activity and increased the subtype selectivity compared with **6e**. Compounds **11ii**, **vi**, **xviii** and **xix**, were too low to detect the affinity for the CCK-A receptor (IC_{50} >10,000).

Derivatives with an *m*-carboxyl group, **11ii**, **vi**, **ix**, **xiii** and **xviii**, retained or showed more potent affinity to the gastrin receptor than L-365,260. Among them, the

Table 2. Receptor antagonistic effects and inhibitory effects on gastric acid secretion

Inhibition Compd	Receptors (IC_{50}, nM)			Gastric acid ED_{50} (mg kg^{-1}) (id)
	Gastrin[a]	CCK-B[a]	CCK-A[a]	
11i	7	140	1350	>0.30[c]
11ii	5	84	>10000[b]	0.09
11iii	4	175	11500	>0.30[c]
11iv	2	8	2900	>0.30[c]
11v	16	180	3600	>0.30[c]
11vi	3	16	4400	>0.30[c]
11vii	11	4	1800	0.30
11viii	2	6	460	0.06
11ix	0.6	2	500	0.20
11x	1	3	600	0.25
11xi	6	115	>10000[b]	>0.30[c]
11xii	15	48	2500	>0.30[c]
11xiii	3	16	4400	>0.30[c]
11xiv	740	>1000[b]	>10000[b]	
11xv	500	>1000[b]	>10000[b]	
11xvi	480	>1000[b]	>10000[b]	
11xvii	0.8	1	1300	0.22
11xviii	13	50	>10000[b]	>0.30[c]
11xix	120	290	>10000[b]	
12i	25	440	3200	
12iv	4	38	3600	0.30
12xii	70	250	3800	
12xiii	7	29	10500	0.40
13iv	6	26	2600	>0.30[c]
14iv	2	3	380	0.30
L-365,260	2.1	15.5	14100	0.104
YM022	0.88	0.89	75	0.107

[a]Binding results are the means of two to four independent determinations.
[b]Full IC_{50} not obtained.
[c]Full ED_{50} not obtained.

sulfide compound **11ix** had the highest affinity to gastrin, though the sulfoxide **11xviii** decreased the potency. The corresponding tetrazole derivative **11xvii** also was equipotent to **11ix**.

Compound **11iv**, with *m*-tetrazole, showed potent affinity to the CCK-B receptor and high subtype selectivity, and three other tetrazole compounds, **12iv**, **13iv** and **14iv**, were examined to investigate the effect of chemical modification at both *N*-1 and *N*-5 positions. Compound **14iv** with a thienylcarbonylmethyl group, retained the potency but the subtype selectivity decreased by one order. Compound **13iv** still showed potent affinity despite lacking the carbonyl moiety.

Derivatives with substituents other than the carboxyl group, **11iii**, **x** and **xi**, also had potent affinity for the gastrin receptor, and **11x** was potent in the in vivo assay (ED_{50} = 0.25 mg kg^{-1}).

These in vitro active compounds **11–14** were assessed in an in vivo Schild inhibition of acid secretion as described above. Compound **11ix** showed an ED_{50} of 0.20 mg kg^{-1}, and the ethyl ester (**11viii**) showed an ED_{50} value of 0.06 mg kg^{-1}, which was more effective than L-365,260 and YM022. The tetrazole derivative, **11xvii**, also had equipotent efficacy to that of **11ix**.

Conclusions

We have discovered that 2,4-dioxo-1,5-benzodiazepine derivatives with a plane of symmetry were potent and subtype-selective CCK-B/gastrin receptor antagonists. The introduction of carbonylmethyl groups to *N*-1 and *N*-5 positions and a carboxyl group onto the benzene ring of the ureido group at the C-3 position caused potent affinity and subtype selectivity for the CCK-B/gastrin receptor. Compound **11viii** potently inhibited pentagastrin-induced gastric acid secretion in anesthetized rats with an ED_{50} value of 0.06 mg kg^{-1}.

Experimental

General method

Mps were not corrected. IR spectra were recorded on a Nicolet 20SXB FT-IR spectrometer. ^1H NMR spectra were recorded on a Varian VXR-200 and VXR-300 FT-^1H NMR spectrometer with tetramethylsilane as an

internal reference. Column chromatography was performed on Merck Kiesel gel 60 in a medium pressure unless otherwise noted. After the extraction of the reaction mixture, the solution was washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. These procedures are simply indicated by the phrase "the extracts were treated as usual" unless otherwise stated.

Diethyl methoxyiminomalonate (2). A solution of diethylketomalonate 1 (51.0 g, 0.293 mmol), *o*-methylhydroxylamine HCl (24.46 g, 0.293 mmol), and pyridine (23.2 g, 0.293 mmol) in ethanol (250 mL) was heated under reflux for 3 h. The solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and the solution was treated as usual. The residue was distilled at 80–85 °C at 0.5 mmHg to give **2** (55.5 g, 93.2%). ^{1}H NMR ($CDCl_3$) δ 1.35 (3H, t, J = 7.1 Hz), 1.35 (3H, t, J = 7.1 Hz), 4.11 (3H, s), 4.36 (2H, q, J = 7.1 Hz), 4.37 (2H, q, J = 7.1 Hz).

3-Methoxyimino-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (3). A mixture of 1N sodium methoxide (162 mL), *o*-phenylenediamine (17.5 g, 162 mmol) and **2** (32.91 g, 162 mmol) was heated under reflux for 5 h. After cooling, the mixture was acidified with 2N HCl (162 mL), and pale yellow crystals (14.3 g, 41%) were collected by filtration. IR ν_{max} (nujol): 1699, 1655, 1460, 1375 cm^{-1}. NMR ($CDCl_3$ + CD_3OD) δ 4.03 (3H, s), 7.10–7.28 (4H, m).

3-Amino-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (4). A solution of **3** (3.78 g, 17.4 mmol) and 10% Pd-C (1.8 g) in methanol (300 mL) was stirred for 15 h under H_2. After removing the catalyst by filtration, the filtrate was concentrated under reduced pressure. The residue was crystallized from methanol to give **4** (2.062 g, 62%). mp 290–291 °C. IR ν_{max} (nujol): 3376, 3287, 1704, 1673, 1563 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 3.75 (1H, s), 7.09–7.25 (4H, m). Anal. calcd for $C_9H_9N_3O_2 \cdot 0.1H_2O$: C, 56.01; H, 4.80; N, 21.77. Found: C, 56.16; H, 4.88; N, 21.64.

3-(*N'*-(*m*-Tolyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (5). A mixture of **4** (1.761 g, 9.21 mmol) and *m*-tolylisocyanate (1.31 g, 10.13 mmol) in dimethylformamide (17 mL) was stirred for 1 h under ice-cooling. To the mixture was added diisopropyl ether (50 mL) and the crystalline precipitates were collected by filtration to give **5** (2.98 g; yield, 99%). mp 300 °C. IR ν_{max} (nujol): 3350, 3301, 3215, 3072, 1714, 1656, 1600, 1562 cm^{-1}. NMR (DMSO-d_6) δ 2.23 (3H, s), 4.63 (1H, d, J = 7.4 Hz), 6.73 (1H, d, J = 7.0 Hz), 6.82 (1H, d, J = 7.4 Hz), 7.31–7.00 (8H, m), 10.77 (2 H, s). Anal. calcd for $C_{17}H_{16}N_4O_3 \cdot 0.1H_2O$: C, 62.61; H, 5.01; N, 17.18. Found: C, 62.45; H, 5.03; N, 17.13.

Chloromethyl cyclopentyl ketone. A quantity of $SOCl_2$ (11.9 g, 10 mmol) was added to cyclopentanecarboxylic acid (5.71 g, 5 mmol) and the mixture was stirred for 2 h at room temperature. After removing excess $SOCl_2$ under reduced pressure, the mixture was distilled at 48–52 °C at 18 mmHg to give acid chloride (5.54 g, 83.5%). A solution of the acid chloride (5.54 g) in ether (5 mL) was added dropwise to a solution of excess diazomethane in ether under ice-cooling. The mixture was stirred for 30 min and concentrated under reduced pressure to about half of its original volume. The solution was added dropwise to concd HCl at −20 °C and the mixture was stirred for 3 h. After adding ice-cold water, the organic layer was treated as usual. The residue was distilled at 88–92 °C at 18 mmHg to give the objective compound (3.53 g, 58.4%). ^{1}H NMR ($CDCl_3$) δ 1.5–2.0 (8H, m), 3.12 (1H, m), 4.17 (2H, s).

2-(Chloroacethyl)furan, 2-(chloroacethyl)thiophene, 4-(chloroacetyl)-1,2-dimethoxybenzene, chloroacetylcyclopropane, and *o*-methylphenacyl chloride were prepared in a similar manner to that described above.

***N*-(Bromoacetyl)pyrrolidine.** A solution of pyrrolidine (3.97 g, 55 mmol) and triethylamine (5.84 g, 57.7 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a solution of bromoacetyl bromide (11.28 g, 91.7 mmol) in CH_2Cl_2 (25 mL) under ice-cooling. The mixture was stirred at 0 °C for 30 min, then at room temperature for 30 min, and poured into ice-cold water. The organic layer was treated as usual.

1,5-Bis-(pyrrolidinocarbonylmethyl)-3-(*N'*-*m*-tolyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)-dione (6a). A mixture of compound **5** (1.06 mmol) and bromoacetylpyrrolidine (2.40 mmol), K_2CO_3 (553 mg, 4.00 mmol) and KI (80 mg, 0.48 mmol) in dimethylformamide was stirred for 15 h, poured into ice-cold water and extracted with ethyl acetate. The extracts were treated as usual. The residue was purified by column chromatography in chloroform:methanol. IR ν_{max} (KBr): 3421, 1694, 1655, 1557 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.70–2.05 (8H, m), 2.25 (3H, s), 3.47 (8H, m), 4.60 (4H, s), 5.23 (1H, m), 6.33 (1H, m), 6.79 (1H, m), 7.04–7.15 (3H, m), 7.22 (1H, s), 7.28 (2H, m), 7.51 (2H, m). Anal. calcd for $C_{29}H_{34}N_6O_5 \cdot 0.6H_20$: C, 62.49; H, 6.36; N, 15.08. Found: C, 62.41; H, 6.42; N, 15.04.

Using the procedure above, the compounds **6a–f, h–p** were prepared from **5**.

1,5-Bis-(*o*-methylphenacyl)-3-{*N'*-(*m*-tolyl)ureido}-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6b). Mp 239–242 °C. IR ν_{max} (KBr): 3408, 3363, 1691, 1670, 1643, 1614, 1600, 1571, 1501 cm^{-1}. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 2.29 (3H, s), 2.41 (6H, s), 5.07 (4H, s), 5.39 (1H, s), 6.80 (1H, m), 7.14 (2H, m), 7.22–7.39 (9H, m), 7.45 (2H, m), 7.65 (2H, d, J = 7.4 Hz). Anal. calcd for $C_{35}H_{32}N_4O_5 \cdot 0.5H_2O$: C, 70.34; H, 5.57; N, 9.37. Found: C, 70.28; H, 5.43; N, 9.52.

1,5-Bis-(3,4-dimethoxyphenacyl)-3-(*N'*-(*m*-tolyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6c). Quantitative yield, mp 266–268 °C. IR ν_{max} (KBr): 3355, 1699, 1678, 1665, 1649, 1614, 1596, 1568 cm^{-1}. ^{1}H NMR (DMSO + CD_3OD) δ 2.24 (3H, s), 3.85 (6H, s), 3.89 (6H, s), 5.15 (1H, d, J = 2 Hz), 5.29 (2H, d, J =18.0 Hz),

5.55 (2H, d, J = 18.0 Hz), 6.73 (1H, d, J = 5.8 Hz), 6.89 (1H, d, J = 8.2 Hz), 7.04–7.16 (4H, m), 7.20 (1H, s), 7.41 (4H, s), 7.52 (2H, d, J = 2.0 Hz), 7.74 (2H, dd, J = 2.8, 8.2 Hz), 9.04 (1H, s). Anal. calcd for $C_{37}H_{36}N_4O_9 \cdot 0.4H_2O$: C, 64.60; H, 5.39; N, 8.14. Found: C, 64.60; H, 5.53; N, 8.10.

1,5-Bis-(2-furylcarbonylmethyl)-3-{*N'*-(*m*-tolyl)ureido}-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6d). Mp 296–298 °C (dec). IR ν_{max} (nujol): 3367, 1706, 1689, 1663, 1644, 1570 cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.22 (3H, s), 5.10 (1H, d, J = 8.2 Hz), 5.12 (2H, d, J = 18.0 Hz), 5.38 (2H, d, J = 18.0 Hz), 6.73 (1H, s), 6.80 (2H, dd, J = 3.6, 1.6 Hz), 7.10 (2H, d, J = 4.4 Hz), 7.19 (1H, s), 7.37–7.55 (4H, m), 7.64 (2H, d, J = 3.6 Hz), 8.10 (2H, d, J = 1.6 Hz), 9.05 (1H, s). Anal. calcd for $C_{29}H_{24}N_4O_7 \cdot 0.5H_2O$: C, 63.38; H, 4.59; N, 10.20. Found: C, 63.28; H, 4.57; N, 10.32.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-tolyl)-ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6e). Yield, 74%; mp 246–248 °C. IR ν_{max} (KBr): 3380, 1700, 1645, 1617, 1565, 1500, 1428 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.79–1,06 (8H, m) 2.06–2.21 (2H, m), 2.22 (3H, s), 4.82 (2H, d, J = 18.2 Hz), 4.99 (1H, d, J = 8.0 Hz), 4.99 (1H, d, J = 18.2 Hz), 6.68–6.79 (1H, m), 6.85 (1H, d, J = 8.0 Hz), 7.09 (1H, d, J = 5.2 Hz), 7.18 (1H, s), 7.28–7.50 (5H, m), 9.03 (1H, s). Anal. calcd for $C_{27}H_{28}N_4O_5 \cdot 0.1H_2O$: C, 66.14; H, 5.80; N, 11.43. Found: C, 66.05; H, 5.80; N, 11.46.

1,5-Bis-(2-triphenylmethyltetrazol-5-ylmethyl)-3-(*N'*-(*m*-tolyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6f). Yield, 49.3%; mp 156–158 °C. IR ν_{max} (KBr): 3411, 1711, 1678, 1612, 1597, 1546 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.27 (3H, s), 4.89 (2H, d, J = 16.4 Hz), 5.05 (2H, d, J = 16.4 Hz), 5.28 (1H, d, J = 7.8 Hz), 6.24 (1H, d, J = 7.8 Hz), 6.70–7.50 (39H, m). Anal. calcd for $C_{59}H_{48}N_{12}O_3 \cdot H_20$: C, 71.50; H, 5.08; N, 16.96. Found: C, 71.28; H, 5.15; N, 17.65.

1,5-Bis-(tetrazol-5-ylmethyl)-3-(*N'*-(*m*-tolyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6g). A 5 mL quantity of 10% HCl was added to a solution of **6f** (353 mg, 0.363 mmol) in methanol (20 mL), and the mixture was stirred for 15 h at room temperature. After the addition of water (150 mL), the mixture was extracted with ethyl acetate. The extracts were washed with water and extracted with 10% aq Na_2CO_3. The aqueous layer was acidified with 10% HCl and extracted with ethyl acetate. The extract was treated as usual. Crystallization from a mixture of methanol and benzene gave **6g** (118 mg, 66.7%). mp 228–230 °C. IR ν_{max} (KBr): 3375, 3274, 1713, 1688, 1647, 1561 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.29 (3H, s), 5.22 (1H, s), 5.37 (2H, d, J = 16.4 Hz), 5.41 (2H, d, J = 16.4 Hz), 6.83 (1H, m), 7.09–7.20 (3H, m), 7.45 (4H, m). Anal. calcd for $C_{21}H_2ON_{12}O_3$: C, 51.64; H, 4.13; N, 34.41. Found: C, 51.51; H, 4.23; N, 34.36.

Disodium 1,5-bis-(tetrazol-5-ylmethyl)-3-(*N'*-(*m*-tolyl)-ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6h). IR ν_{max} (KBr): 3380, 1700, 1669, 1613, 1559 cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.21 (3H, s), 4.83 (2H, d, J = 15.6 Hz), 4.92 (1H, d, J = 8.2 Hz), 5.24(2H, d, J = 15.6 Hz), 6.70 (1H, d, J = 5.0 Hz), 6.80 (1H, d, J = 8.2 Hz), 7.01–7.13, (2H m), 7.18 (1H, s), 7.29 (2H, m), 7.88 (2H, m), 9.07 (1H, s). Anal. calcd for $C_{21}H_{18}N_{12}O_3Na_2 \cdot 1.6H20$: C, 44.94; H, 3.81; N, 29.95; Na, 8.19. Found: C, 44.80; H, 3.39; N, 30.09; Na, 8.26.

1,5-Bis-allyl-3-{*N'*-(*m*-tolyl)ureido}-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6i). Mp 210–211 °C. IR ν_{max} (nujol): 3327, 1702, 1666, 1641, 1561 cm^{-1}. NMR (DMSO-d_6) δ 2.22 (3H, s), 4.45 (2H, dd, J = 15.9, 6.0 Hz), 4.67 (2H, dd, J = 15.9, 6.0 Hz), 4.90 (1H, d, J = 7.6 Hz), 5.04–5.10 (2H, m), 5.14 (2H, d, J = 3.0 Hz), 5.59–5.82 (2H, m), 6.68–6.78 (1H, m), 6.88 (1H, d, J = 7.6 Hz), 7.08–7.14 (2H, m). 7.17 (1H, s), 7.35–7.48 (2H, m), 7.55–7.68 (2H, m), 9.08 (1H, s). Anal. calcd for $C_{23}H_{24}N_4O_3 \cdot 0.3H_20$: C, 67.40; H, 6.05; N, 13.67. Found: C, 67.50; H, 6.02; N, 13.65.

1,5-Bis-(cyclopropylmethyl)-3-{*N'*-(*m*-tolyl)ureido}-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6j). Mp 221–222 °C. IR ν_{max} (nujol): 3315, 1693, 1643, 1611 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.09–0.18 (4H, m), 0.23–0.37(4H, m), 0.72–0.92 (2H, m), 2.22 (3H, s), 3.66 (2H, dd, J = 14.2, 6.8 Hz), 4.16 (2H, dd, J = 14.2, 6.8 Hz), 4.79 (1H, d, J = 7.6 Hz), 6.67–6.76 (1H, m), 6.84 (1H, d, J = 7.8 Hz), 7.10 (2H, dd, J = 3.0, 1.0 Hz), 7.18 (1H, s), 7.37–7.51 (2H, m), 7.66–7.78 (2H, m), 9.09 (1H, s). Anal. calcd for $C_{25}H_{28}N_4O_3 \cdot 0.4H_20$: C, 68.29; H, 6.60; N, 12.74. Found: C, 68.40; H, 6.50; N, 12.81.

1,5-Bis-(*o*-methylbenzyl)-3-{*N'*-(*m*-tolyl)ureido}-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6k). Mp 225–227 °C. IR ν_{max} (nujol): 3334, 1701, 1688, 1641 cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.12 (6H, s), 2.25 (3H, s), 4.99 (2H, d, J = 16.4 Hz), 5.09 (2H, d, J = 16.4 Hz), 5.16 (1H, d, J = 7.4 Hz), 6.75 (1H, d, J = 6.2 Hz), 6.88–7.23 (12H, m), 7.28–7.37 (2H, m), 7.45–7.56 (2H, m), 9.11 (1H, s). Anal. calcd for $C_{33}H_{32}N_4O_3$: C, 74.41; H, 6.06; N, 10.52. Found: C, 74.19; H, 6.15; N, 10.34.

1,5-Bis-(*m*-tert-butoxycarbonylaminophenylmethyl)-3-(*N'*-(*m*-tolyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6l). Yield, 65.3%.IR ν_{max} (KBr): 3413, 1703, 1675, 1612, 1547 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.50 (18H, s), 2.10 (3H, s), 4.90 (4H, s), 5.47 (1H, d, J = 8.2 Hz), 6.62 (2H, t, J = 7.4 Hz), 6.70 (2H, d, J = 8.0 Hz), 6.87 (1H, d, J = 7.8 Hz), 6.87 (1H, d, J = 7.8 Hz), 6.92 (1H, s), 7.04 (11H, m), 7.61 (2H, d, J = 8.2 Hz), 7.60 (1H, s). Anal. calcd for $C_{41}H_{46}N_6O_7 \cdot 0.5H_20$: C, 66.20; H, 5.37; N, 11.30. Found: C, 66.31; H, 6.51; N, 11.08.

1,5-Bis-(*m*-anilinomethyl)-3-(*N'*-(*m*-tolyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6m). Mp 176–179 °C, 99.0% yield. IR ν_{max} (KBr): 3416, 2864, 2591, 1697, 1663, 1603, 1557 cm^{-1}. ^1H NMR (CD_3OD) δ 2.29 (3H, s), 5.06 (2H, d, J = 16.2 Hz), 5.25 (1H, s), 5.25 (2H, d, J = 16.2 Hz), 6.84 (1H, m), 7.11–7.18 (3H, m),

7.25–7.56 (12H, m). Anal. calcd for $C_{31}H_{32}N_6O_3Cl\cdot 2H_2O$: C, 59.52; H, 5.48; N, 13.43; Cl, 11.33. Found: C, 59.43; H, 5.69; N, 13.16; Cl, 11.54.

1,5-Bis-(*tert*-butoxycarbonylmethyl)-3-(*N'*-(*m*-tolyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6n). Yield 37.3%; mp 218–219 °C. IR ν_{max} (KBr): 3370, 1742, 1708, 1653, 1615, 1570, 1500, 1422, 1370, 1225, 1155 cm^{-1}. ^1H NMR ($CDCl_3$–CD_3OD) δ 1.44 (18H, s), 2.28, (3H, s), 4.46 (4H, q, J = 9.8 Hz), 5.30 (1H, s), 6.16–6.30 (1H, br.s), 6.71–6.88 (2H, m), 7.03–7.23 (3H, m). 7.36 (4H, m). Anal. calcd for $C_{29}H_{36}N_4O_7\cdot 2H_20$: C, 62.62; H, 6.60; N, 10.07. Found: C, 62.59; H, 6.60; N, 10.10.

1,5-Bis-(2-hydroxyethyl)-3-(*N'*-(*m*-tolyl)-ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6o). Mp 221–224 °C. IR ν_{max} (KBr): 3437, 3387, 1701, 1629, 1561 cm^{-1}. NMR ($CDCl_3$ + CD_3OD) δ 2.28 (3H, s), 3.59 (2H, m), 3.76 (4H, m), 4.61 (2H, m), 5.12 (1H, s), 6.80 (1H, m), 7.12 (2H, m), 7.21 (1H, s), 7.43 (4H, m). Anal. calcd for $C_{21}H_{24}N_4O_3\cdot 0.2H_20$: C, 60.63; H, 5.91; N, 13.47. Found: C, 60.53; H, 5.92; N, 13.55.

1,5-Bis-(carboxymethyl)-3-(*N'*-(*m*-tolyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (6p). A Jones' reagent (3M, 1 mL) was added dropwise to a solution of **6o** (193 mg, 0.468 mmol) in acetone (24 mL), and the mixture was stirred for 2 h at 55 °C. After cooling, water was added. The mixture was extracted with chloroform:methanol (4:1) to obtain the carboxylic acid **6p** (166 mg, 80.6%). ^1H NMR ($CDCl_3$ + CD_3OD) δ 2.27 (3H, s), 4.50 (2H, d, J = 17.6 Hz), 4.55 (2H,d, J = 17.6 Hz), 5.30 (1H, s), 6.80 (1H, m), 7.11 (2H, m), 7.19 (1H, m), 7.41 (4H, m).

3-(*tert*-Butoxycarbonylamino)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (7). Di-*tert*-butyldicarbonate (5.62 g, 25.8 mmol) was added to a suspension of **4** (3.27 g, 17.1 mmol) in THF (300 mL). The mixture was stirred for 24 h at room temperature and concentrated. A mixture (200 mL) of CH_2Cl_2:methanol (9:1) and water (50 mL) was added to the residue and the mixture was stirred for 10 min. The organic layer was treated as usual. Crystallization from CH_2Cl_2/methanol and diisopropylether yields **7** (4.5 g, yield 90%) as colorless crystals, mp 243–244 °C. ^1H NMR (DMSO-d_6) δ 1.38 (9H, m), 4.51 (1H, d, J = 8.2 Hz), 6.48 (1H, d, J = 8.2 Hz), 7.14–7.31 (4H, m), 10.54–10.93 (2H, br s). Anal. calcd for $C_{14}H_{17}N_3O_4$: C, 57.72; H, 5.88; N, 14.43. Found: C, 57.45; H, 5.88; N, 14.36.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*tert*-butoxycarbonylamino)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (8a). A suspension of **7** (2.039 g, 7 mmol), cyclopropylcarbonylmethylchloride (2.488 g, 21 mmol), K_2CO_3 (2.902 g, 21 mmol), and KI (174 mg, 1.05 mmol) in dimethylformamide (20 mL) was stirred for 15 h at room temperature. The mixture was concentrated under reduced pressure and chromatographed in toluene:ethyl acetate (2:1) to give **8a** (3.188 g; yield, 100%) as a foam. IR ν_{max} (KBr): 3445, 1700, 1658, 1503, 1450, 1320 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.81–1.06 (8H, m) 1.35 (9H, s), 2.05–2.22 (2H, m), 4.82 (2H, d, J = 18.4 Hz), 4.85 (1H, d, J = 8.2 Hz), 4.96 (2H, d, J = 18.4 Hz), 6.73 (1H, d, J = 8.2 Hz), 7.27–7.45 (4H, m). Anal. calcd for $C_{24}H_{29}N_3O_6\cdot 0.2H_2O$: C, 62.79; H, 6.45; N, 9.15. Found: C, 62.92; H, 6.44; N, 8.94.

Using the procedure above, the compounds **8b–d** and **9** were prepared from **7** and **3**.

1,5-Bis-(pyrrolidinocarbonylmethyl)-3-(*tert*-butoxycarbonylamino)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (8b). Mp 137–139 °C. IR ν_{max} (KBr): 3440, 1700, 1503, 1420 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.36 (9H, s), 1.65–1.96 (8H, m), 3.23–3.38 (4H, m), 3.39–3.52 (4H, m), 4.47 (2H, d, J = 16.6 Hz), 4.68 (2H, d, J = 16.6 Hz), 4.83 (1H, d, J = 8.2 Hz), 6.57 (1H, d, J = 8.2 Hz), 7.32–7.46 (2H, m), 7.47–7.57 (2H, m). Anal. calcd for $C_{26}H_{35}N_5O_6\cdot 0.7H_2O$: C, 59.35; H, 6.97; N, 13.31. Found: C, 59.35; H, 6.84; N, 13.14.

1,5-Bis-(thienylcarbonylmethyl)-3-(tert-butoxycarbonylamino)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (8d). Mp 132–135 °C. IR ν_{max} (KBr): 3435, 1703, 1672, 1503, 1419 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.36 (9H, s), 4.96 (1H, d, J = 8.4 Hz), 5.27 (2H, d, J = 18.0 Hz), 5.52 (2H, d, J = 18.0 Hz), 6.82 (1H, d, J = 8.4 Hz), 7.28–7,36 (2H, m), 7.45 (4H, d, J = 2.0 Hz), 8.10–8.18 (4H, m). Anal. calcd for $C_{26}H_{25}N_3O_6S_2\cdot 0.2H_2O$: C, 57.49; H, 4.71; N, 7.74; S, 11.80. Found: C, 57.54; H, 4.81; N, 7.71; S, 11.69.

1,5-Bis-(cyclopropylmethyl)-3-(*tert*-butoxycarbonylamino)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (8c). Mp 156–157 °C. IR ν_{max} (KBr): 3430, 3370, 1695, 1500, 1419 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.04–0.17 (4H, m), 0.21–0.36 (4H, m), 0.69–0.89 (2H, m), 1.35 (9H, s), 3.64 (2H, dd, J = 14.6, 6.8 Hz), 4.14 (2H, dd, J = 14.6, 6.8 Hz), 4.65 (1H, d, J = 8.2 Hz), 6.45 (1H, d, J = 8.2 Hz), 7.37–7.49 (2H, m), 7.63–7.77 (2H, m). Anal. calcd for $C_{26}H_{35}N_5O_6$: C, 59.35; H, 6.97; N, 13.31. Found: C, 59.35; H, 6.84; N, 13.14.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(methoxyimino)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (9). Mp 228–230 °C, 87.1% yield. IR ν_{max} (KBr): 3443, 1714, 1678, 1660, 1598, 1503 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.93–1.08 (4H, m),1.15 (4H, m), 2.03 (2H, m), 3.96 (3H, s), 4.78 (2H, d, J = 17.6 Hz), 4.90 (1H, d, J = 17.6 Hz), 4.92 (2H, d, J = 17.6 Hz), 7.17–7.33 (4H, m). Anal. calcd for $C_{20}H_{21}N_3O_5$: C, 62.65; H, 5.52; N, 10.96. Found: C, 62.41; H, 5.62; N, 11.02.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-amino-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (10a). (1) A solution of 4N HCl in ethyl acetate (14 mL) was added under ice-cooling to a solution of **8a** (3.188 g) in ethyl acetate (16 mL). After stirring the reaction mixture for 15 h at room temperature, crystalline preparations were collected by filtration. The crystals were dissolved into CH_2Cl_2:methanol (5:1) and treated as usual. Crystallization from CH_2Cl_2, methanol, and diisopropylether yields **10a** (2.092 g; yield, 84%), mp 237–238 °C. IR ν_{max}

(KBr): 3375, 1700, 1667, 1600, 1505, 1416, 1389 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.79–1.03 (8H, m), 1.82–2.00 (2H, br.s), 2.05–2.21 (2H, m), 4.08 (1H, s), 4.74 (2H, d, J = 18.2 Hz), 4.96 (2H, d, J = 18.2 Hz), 7.19–7.30 (2H, m), 7.30–7.40 (2H, m). Anal. calcd for $C_{19}H_{21}N_3O_4 \cdot 0.3H_2O$: C, 63.25; H, 6.03; N, 11.65. Found: C, 63.35; H, 5.92; N, 11.65.

(2) A 1.5 g quantity of 10% Pd-C was added to a suspension of **9** (3.34 g, 8.71 mmol) in methanol (320 mL) and the mixture was stirred for 20 h under H_2. Organic substances were dissolved in chloroform and the catalysts were filtered off. The residue obtained by distilling the solvent under reduced pressure was dissolved in chloroform (100 mL) and methanol (300 mL), and again subjected to the reduction with 10% Pd–C catalyst for 20 h. After removing the catalysts by filtration, the solvent was distilled under reduced pressure and the residue was re-precipitated from methanol–ethyl acetate to give **10a** (3.05 g, 98.5 %).

Using the procedure of (1), the following compounds, 10b–d were prepared from 8b–d

1,5-Bis-(pyrrolidinocarbonylmethyl)-3-amino-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (10b). Mp 246–247 °C. IR ν_{max} (KBr): 3445, 1700, 1655, 1500, 1450, 1320 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.63–2.00 (10H, m), 3.23–3.38 (4H, m), 3.40–3.53 (4H, m), 4.04 (1H, s), 4.39 (2H, d, J = 16.6 Hz), 4.69 (2H, d, J = 16.6 Hz), 7.29–7.40 (2H, m), 7.40–7.51(2H, m). Anal. calcd for $C_{21}H_{27}N_5O_4 \cdot 0.7H_2O$: C, 59.20; H, 6.72; N, 16.44. Found: C, 59.38; H, 6.46; N, 16.20.

1,5-Bis-(thienylcarbonylmethyl)-3-amino-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (10d). Mp 208–209 °C. IR ν_{max} (KBr): 3450, 1700, 1679, 1662, 1585, 1502, 1419, 1241 cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.96 (2H, s), 4.21 (1H, s), 5.18 (2H, d, J = 18.0 Hz), 5.51 (2H, d, J = 18.0 Hz), 7.26–7.45 (6H, m), 8.08–8.20 (4H, m). Anal. calcd for $C_{21}H_{17}N_3O_4S_2$: C, 57.39; H, 3.90; N, 9.56; S, 14.59. Found: C, 57.28; H, 4.03; N, 9.40; S, 14.40.

1,5-Bis-(cyclopropylmethyl)-3-amino-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (10c). Mp 147–148 °C. IR ν_{max} (KBr): 3380, 1696, 1660, 1600, 1500, 1422 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.04–0.17 (4H, m), 0.22–0.35 (4H, m), 0.70–0.90 (2H, m), 1.89 (2H, br.s), 3.63 (2H, dd, J = 14.2, 6.6 Hz), 3.89 (1H, s), 4.12 (2H, dd, J = 14.2, 6.6 Hz), 7.32–7.43 (2H, m), 7.58–7.71 (2H, m). Anal. calcd for $C_{17}H_{21}N_3O_2$: C, 68.21; H, 7.07; N, 14.04. Found: C, 68.20; H, 7.12; N, 13.96.

Ethyl-3-(*N*-*tert*-butoxycarbonylamino)phenylthioacetate. Di-*tert*-butyldicarbonate (3.50 g, 16.04 mmol), and then 5% aq $NaHCO_3$ solution (10 mL) were added under ice-cooling to a solution of *m*-aminobenzenethiol (2.0 g, 15.9 mmol) in acetone (20 mL). The mixture was stirred at 0 ·C for 30 min, and then at room temperature for 15 h. After the addition of ethyl acetate, the reaction mixture was treated as usual. The residue was purified by column chromatography in hexane:toluene, 2:1 to give the product (3.09 g, 85.8%). A 10 g quantity of K_2CO_3 and 2.35 g of ethyl bromoacetate were added, with stirring, to a solution of the compound (3.09 g) in acetone (50 mL). After the reaction mixture was stirred for three days, ether was added and the insoluble substances were removed by filtration. The filtrate was distilled under reduced pressure. The residue was purified by column chromatography (hexane:ethyl acetate, 3:1 to 2:1) and crystallization from ether/hexane to give the titled compound (2.4 g, 56.2%), mp 70–71 °C. IR ν_{max} (KBr): 3424, 3353, 1717, 1599, 1538 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.3 Hz), 1.51 (9H, s), 3.64 (2H, s), 4.18 (2H, q, J = 7.3 Hz), 6.50 (1H, s), 7.00–7.11 (1H, m), 7.15–7.25 (2H, m), 7.47 (1H, m). Anal. calcd for $C_{15}H_{28}NO_4S$: C, 57.86; H, 6.80; N, 4.50; S, 10.30. Found: C, 57.61; H, 6.82; N, 4.67; S, 10.27.

Ethyl-3-aminophenylthioacetate. The above mentioned BOC compound (1.255 g) was treated by the same procedure as described for the preparation of **10a**. The crude amine (846 mg) was used in the next step without further purification. ^1H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.1 Hz), 3.62 (2H, s), 4.18 (2H, q, J = 7.1 Hz), 6.54 (1H, ddd, J = 1.0, 2.3, 8.0 Hz), 6.72–6.81 (2H, m), 7.08 (1H, t, J = 7.7 Hz).

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-carboethoxyphenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)-dione (11i). Triphosgene (126 mg, 0.424 mmol) and triethylamine (354 mL, 2.54 mmol) were added successively to a solution of methyl 3-aminobenzoate (200 mg, 1.21 mmol) in tetrahydrofuran (10 mL). After stirring for 15 min, compound **10d** (300 mg, 0.844 mmol) was added and the stirring was continued for another 3 h. The mixture was partitioned between a mixture of CH_2Cl_2:methanol (5:1) and water. The extracts were treated as usual. The residue was purified by column chromatography in toluene:ethyl acetate, 1:1, and recrystallized from a mixture of CH_2Cl_2, methanol, and diisopropylether to give **11i** (320 mg; yield, 69%), mp 222–223 °C. IR ν_{max} (KBr): 3400, 1708, 1599, 1562, 1503, 1425 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.79–1.04 (8H, m), 1.29 (3H, t, J = 7.0 Hz), 2.06–2.22 (2H, m), 4.28 (2H, q, J = 7.0 Hz), 4.83 (2H, d, J = 18.0 Hz), 5.00 (1H, d, J = 7.8 Hz), 5.00 (2H, d, J = 18.0 Hz), 6.89 (1H, d, J = 7.8 Hz), 7.27–7.60 (7H, m), 8.60 (1H, s), 9.39 (1H, s). Anal. calcd for $C_{29}H_{30}N_4O_7 \cdot 0.3H_2O$: C, 63.10; H, 5.59; N, 10.15. Found: C, 63.05; H, 5.61; N, 10.28.

Using the procedure above, the compounds **11–14** were prepared from **10a–d**.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-carboxyphenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11ii). Mp 274–276 °C. IR ν_{max} (KBr): 3405, 1702, 1561, 1500, 1431 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.75–1.05 (8H, m), 2.06–2.24 (2H, m), 4.83 (2H, d, J = 18.2 Hz), 5.00 (1H, d, J = 7.8 Hz), 5.00 (2H, d, J = 18.2 Hz), 6.88 (1H, d, J = 7.8 Hz), 7.27–7.55 (7H, m), 8.00 (1H, s), 9.30 (1H, s). Anal. calcd for $C_{27}H_{26}N_4O_7 \cdot 0.5H_2O$: C, 61.47; H, 5.16; N, 10.62. Found: C, 61.47; H, 5,15; N, 10.62.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(isopropylsulfonylaminocarbonyl)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11iii). Compound **11iii** was prepared using 3-isopropylsulfonylaniline.[11] Mp 193–194 °C. IR ν_{max} (KBr): 3380, 1700, 1602, 1553, 1503, 1432 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.80–1.05 (8H, m), 1.22 (6H, d, J = 6.8 Hz), 2.06–2.23 (2H, m), 3.55–3.76 (1H, br.s), 4.83 (2H, d, J = 18.2 Hz), 5.00 (1H, d, J = 8.0 Hz), 5.00 (2H, d, J = 18.2 Hz), 6.87 (1H, d, J = 8.0 Hz), 7.21–7,56 (7H, m), 7.85 (1H, s), 9.33 (1H, s). Anal. calcd for $C_{30}H_{33}N_5O_8S_2 \cdot 2H_2O$: C, 54.32; H, 5.68; N, 10.56; S, 4.83. Found: C, 54.16; H, 5.58; N, 10.69; S, 4.88.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(tetrazoyl)-phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11iv). 3-Amino-(1*H*-tetrazol-5-yl)benzene (disclosed in EP 0 508 796 A1) was treated by the same procedure described in the preparation of **11i**; 48% yield as colorless crystals, mp 213–215 °C. IR ν_{max} (KBr): 3400, 1685, 1660, 1600, 1550, 1502, 1433 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.72–1.04 (8H, m), 2.06–2.25 (2H, m), 4.84 (2H, d, J = 18.4 Hz), 5.01 (2H, d, J = 18.4 Hz), 5.03 (1H, d, J = 7.8 Hz), 6.93 (1H, d, J = 7.8 Hz), 7.26–7.60 (8H, m), 8.04 (1H, s), 9.31 (1H, s). Anal. calcd for $C_{27}H_{26}N_8O_5 \cdot 1.5H_2O$: C, 56.94; H, 5.13; N, 19.67. Found: C, 56.72; H, 5.04; N, 19.38.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(carboethoxymethoxy)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11v).

(1) Ethyl-3-(*N*-*tert*-butyloxycarbonylamino)-phenyloxyacetate. Di-*tert*-butyldicarbonate (4.578 g, 21 mmol) was added to a solution of 3-aminophenol (2.18 g, 20 mmol) in THF (10 mL), and the mixture was stirred for 15 h at room temperature. The residue obtained by concentrating the reaction mixture was purified by chromatography in hexane:ethyl acetate (5:1). Ethyl-2-bromoacetate (2.44 mL, 22 mmol), KI (183 mg, 1.1 mmol), and K_2CO_3 (3.04 g, 22 mmol) were added to a solution of the resulting oily residue of 3-(*N*-*tert*-butyloxycarbonylamino)phenol in dimethylformamide, and the mixture was stirred for 15 h at room temperature. The mixture was concentrated under reduced pressure and the residue was partitioned between a mixture of ethyl acetate and water. The organic layer was treated as usual. The crude product was purified by chromatography in toluene:ethyl acetate (9:1) to give the titled compound as colorless oil. ^1H NMR ($CDCl_3$) δ 1.22 (3H, t, J = 7.4 Hz), 1.47 (9H, s), 4.17 (2H, q, J = 7.4 Hz), 4.70 (2H, s), 6.47–6.57 (1H, m), 6.99–7.21(3H, m), 9.34 (1H, s).

(2) Ethyl-3-aminophenyloxyacetate. Deprotection was carried out by the same procedure described for the preparation of **10a** from **8a** in 45% yield. ^1H NMR ($CDCl_3$) δ 1.30 (3H, t, J = 7.4Hz), 4.27 (2H, q, J = 7.4 Hz), 4.57 (2H, s), 6.23–6.38 (3H, m), 7.00–7.11 (1H,m).

(3) 11v. Mp 205–206 °C. IR ν_{max} (KBr): 3370, 1760, 1700, 1642, 1608, 1602, 1562, 1500, 1455, 1428 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.77–1.08 (8H, m), 1.19 (3H, t, J = 7.0 Hz), 2.06–2.23 (2H, m), 4.15 (2H, q, J = 7.0 Hz), 4.86 (2H, s), 4.83 (2 H, d, J = 18.0 Hz), 4.99 (1H, d, J = 8.0 Hz), 5.00 (2H, d, J = 18.0 Hz), 6.46 (1H, dd, J = 8.2, 2.4 Hz), 6.79–6.92 (2H, m), 6.84 (1H, s), 7.12 (1H, t, J = 8.2 Hz), 7.27–7.50 (4H, m), 9.16 (1H, s). Anal. calcd for $C_{30}H_{32}N_4O_8 \cdot 0.3H_2O$: C, 61.91; H, 5.65; N, 9.63. Found: C, 61.91; H, 5.66; N, 9.61.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(carboxymethoxy)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11vi). Mp 257–259 °C. IR ν_{max} (KBr): 3400, 1755, 1698, 1650, 1620, 1565, 1425 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.77–1.03 (8H, m), 2.06–2.22 (2H, m), 4.57 (2 H, s) 4.86 (2H, s), 4.82 (2H, d, J = 18.2 Hz), 4.99 (1H, d, J = 8.2 Hz), 4.99 (2H, d, J = 18.2 Hz), 6.45 (1H, dd, J = 6.8, 2.0 Hz), 6.77–6.90 (2H, m) 7.06 (1H, br.s.), 7.11 (1H, t, J = 8.0 Hz), 7.28–7,49 (4H, m), 9.15 (1H, s). Anal. calcd for $C_{28}H_{28}N_4O_8 \cdot 0.7H_2O$: C, 59.93; H, 5.28; N, 9.98. Found: C, 59.93; H, 5.12; N, 9.97.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(tetrazoylmethoxy)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11vii).

(1) 3-Nitrophenoxyacetonitrile. A 6.944 g quantity of 97% bromoacetonitrile (55 mmol), KI (457 mg, 2.75 mmol), and K_2CO_3 (7.60 g, 55 mmol) were added to a solution of 3-nitrophenol (6.955 g, 50 mmol) in dimethylformamide, and the mixture was stirred for 15 h at room temperature. The mixture was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate and water. The organic layer was treated as usual. The residue was purified by chromatography in toluene:ethyl acetate (9:1) to give the titled compound (7.128 g; yield, 80%) as colorless crystals.

(2) 3-(Tetrazo-5-yl)methoxyaniline. 3-Nitrophenoxyacetonitrile was treated with NaN_3 to convert the nitrile group into a tetrazole group, followed by catalytic reduction, according to the method described in patent.[11] ^1H NMR (CD3OD) δ 5.36 (2H, s), 6.39–6.48 (3H, m), 7.05 (1H, t, J =0.8Hz).

(3) 11I. Mp 203–204 °C. IR ν_{max} (KBr): 3380, 1700, 1605, 1558, 1502, 1430 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.78–1.06 (8H, m), 2.06–2.23 (2H, m), 4.80 (2H, d, J = 18.2 Hz), 4.99 (1H, d, J = 8.0 Hz), 5.00 (2H, d, J = 18.2 Hz), 5.40 (2H, s) 6.58 (1H, m), 6.81–6.94 (2H, m), 7.10–7.22 (2H, m), 7.26–7,49 (4H, m), 9.18 (1H, s). Anal. calcd for $C_{28}H_{28}N_8O_6 \cdot 0.5H_2O$: C, 57.83; H, 5.03; N, 19.27. Found: C, 57.90; H, 4.98; N, 19.24.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(carboethoxymethylthio)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11viii). Yield 82.6%, mp 193–196 °C. IR ν_{max} (KBr): 3379, 1716, 1699, 1649, 1609, 1587, 1545 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.87–1.16 (8H, m), 1.20 (3H, t, J = 7.2 Hz), 2.00 (2H, m), 3.62 (2H, s), 4.14 (2H, q, J = 7.2 Hz), 4.77 (2H, d, J = 18.0 Hz), 4.93 (2H, d, J = 18.0 Hz), 5.34 (1H, d, J = 7.4 Hz), 6.50 (1H, d, J

= 7.8 Hz), 6.98 (1H, dt, J = 2.2, 6.6 Hz), 7.05–7.18 (2H, m), 7.20–7.36 (5H, m), 7.39 (1H,m). Anal. calcd for $C_{30}H_{32}N_4O_7S \cdot 0.2H_2O$: C, 60.43; H, 5.48; N, 9.40; S, 5.38. Found: C, 60.50; H, 5.55; N, 9.53; S, 5.34.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(carboxymethylthio)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)-dione (11ix). IR ν_{max} (KBr): 3382, 1705, 1673, 1638, 1601, 1589, 1547 cm^{-1}. ^1H NMR ($CDCl_3$ + CD_3OD) δ 0.87–1.20 (8H, m),2.02 (2H, m), 7.00 (1H, dt, J = 1.8, 7.4 Hz), 7.14 (1H, t, J = 7.6 Hz), 7.23–7.39 (4H, m), 7.42 (1H, t, J = 2.0 Hz). Anal. calcd for $C_{28}H_{28}N_4O_7S \cdot 0.5H_2O$: C, 58.68; H, 5.10; N, 9.77; S, 5.59. Found: C, 58.49; H, 5.26; N, 10.06; S, 5.63.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(5-keto-1,2,4-oxadiazol-3-yl)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11x). This was prepared using *m*-(5-keto-1,2,4-oxadiazolyl)phenylisocyanate[11] in 53.4 % yield. IR ν_{max} (KBr): 3426, 1789, 1758, 1700,1640 cm^{-1}. ^1H NMR ($CDCl_3$ + CD_3OD) δ 0.90–1.13 (6H, m), 1.19–1.33 (2H, m), 2.03 (2H, m), 4.61 (2H, d, J = 18.0 Hz), 5.17 (2H, d, J = 18.0 Hz), 5.45 (1H, d, J = 7.8 Hz), 6.57 (1H, s), 6.94 (1H, dd, J = 2.8, 8.0 Hz), 6.99 (1H, d, J = 8.0 Hz), 7.20 (2H, m),7.33 (1H, m), 7.40 (2H, m), 7.50 (1H, d, J = 8.0 Hz), 8.28 (1H, s), 10.98 (1H, s). Anal. calcd for $C_{28}H_{26}N_6O_7S \cdot 0.5H_2O$: C, 59.26; H, 4.79; N, 14.8. Found: C, 59.25; H, 4.99; N, 14.92.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(acetylsulfamoyl)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11xi). Compound **11xi** was prepared using *m*-(acetylsulfamoyl)phenylisocyanate[11] in 31.0% yield. IR ν_{max} (KBr): 3392, 1699, 1664, 1646, 1597, 1553 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.80–1.04 (8H, m), 1.86 (3H, s), 2.15 (2H, m), 4.85 (2H, d, J = 18.0 Hz), 4.99 (2H, d, J = 7.8 Hz), 4.99 (1H, d, J = 18.0 Hz), 6.91 (1H, d, J = 7.8 Hz), 7.27–7.53 (8H, m), 7.99 (1H, s), 9.49 (1H, s). Anal. calcd for $C_{28}H_{29}N_5O_8S \cdot 1.7H_2O$: C, 53.70; H, 5.21; N, 11.18; S, 5.12. Found: C, 53.59; H, 5.08; N, 11.37; S, 5.30.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(carbomethoxymethyl)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11xii).

(1) 3-(*N'*-(*m*-(carbomethoxymethyl)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione. This was prepared using *m*-carbomethoxymethylphenylisocyanate in 86.8% yield, mp >300 °C. IR ν_{max} (KBr): 3372, 1717, 1678, 1607, 1600, 1559, 1501 cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.60 (5H, s), 4.63 (1H, d, J = 7.4 Hz), 6.80 (1H, d, J = 6.6 Hz), 6.84 (1H, d, J = 7.4 Hz), 7.11–7.31 (7H, m), 9.14 (1H, s), 10.77 (2H, s). Anal. calcd for $C_{19}H_{18}N_4O_5 \cdot 0.3H_2O$: C, 58.58; H, 4.83; N, 14.45. Found: C, 58.70; H, 4.92; N, 14.56.

(2) 11xii. Mp 199–202 °C. IR ν_{max} (KBr): 3382, 1699, 1613, 1597, 1558 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.97 (4H, m), 1.10 (4H, m), 2.00 (2H, m), 3.54 (2H, s), 3.66 (3H, s), 4.79 (2H, d, J = 18.0 Hz), 4.91 (2H, d, J = 18.0 Hz), 5.33 (1H, d, J = 7.4 Hz), 6.42 (1H, d, J = 7.8 Hz), 6.89 (1H, dt, J = 2.0, 6.2 Hz), 7.10–7.36 (8H, m). Anal. calcd for $C_{29}H_{30}N_4O_7$: C, 63.73; H, 5.53; N, 10.25. Found: C, 63.54; H, 5.62; N, 10.07.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(carbomethyl)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11xiii). Yield, 59.3%; mp 209–211 °C. IR ν_{max} (KBr): 3385, 1700, 1665, 1647, 1616, 1597, 1588, 1501 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.80–1.08 (8H, m), 2.15 (2H, qui, J = 6.1 Hz), 3.47 (2H, s), 4.84 (2H, d, J = 18.0 Hz), 4.98 (2H, d, J = 18.0 Hz), 4.99 (1 H, d, J = 8.0 Hz), 6.80 (1H, dt, J = 2.0, 6.8 Hz), 6.85 (1H, d, J = 8.0 Hz), 7.09–7.47 (7H, m), 9.12 (1H, s), 12.30 (1H, br s). Anal. calcd for $C_{28}H_{28}N_4O_7$: C, 62.31; H, 5.38; N, 10.38. Found: C, 62.59; H, 5.47; N, 10.10.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*p*-(carbomethoxy)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11xiv). Mp 278–279 °C. IR ν_{max} (KBr): 3350, 1721, 1680, 1650, 1600, 1539, 1500, 1435 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.78–1.04 (8H, m), 2.06–2.22 (2H, m), 3.79 (3H, s), 4.83 (2H, d, J = 18.4 Hz), 4.99 (2H, d, J = 8.0 Hz), 5.00 (2H, d, J = 18.4 Hz), 7.03 (1H, d, J = 7.6 Hz), 7.27–7.53 (4H, m), 7.45 (2H, d, J = 8.6 Hz), 7.83 (2H, d, J = 8.6 Hz), 9.54 (1H, s). Anal. calcd for $C_{28}H_{28}N_4O_7 \cdot 0.4H_2O$: C, 62.31; H, 5.38; N, 10.38. Found: C, 62.23; H, 5.27; N, 10.40.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*'-(*p*-(carboxy)-phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11xv). Mp 261–262 °C. IR ν_{max} (KBr): 3400, 1695, 1600, 1545, 1503, 1432 cm^{-1}. NMR (DMSO-d_6) δ 0.78–1.05 (8H, m), 2.04–2.23 (2H, m), 4.83 (2H, d, J = 18.4 Hz), 5.00 (1H, d, J = 7.8 Hz), 5.00 (2H, d, J = 18.4 Hz), 7.00 (1H, d, J = 7.8 Hz), 7.26–7.51(4H, m), 7.43 (2H, d, J = 8.8 Hz), 7.81 (2H, d, J = 8.8 Hz), 9.49 (1H, s). Anal. calcd for $C_{27}H_{26}N_4O_7 \cdot 0.3H_2O$: C, 61.19; H, 5.12; N, 10.69. Found: C, 61.85; H, 5.07; N, 10.73.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*p*-cyanophenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11xvi). Mp 260–262 °C. IR ν_{max} (KBr): 3380, 2220, 1703, 1670, 1600, 1540, 1500, 1430 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.76–1.05 (8H, m), 2.06–2.24 (2H, m), 4.83 (2H, d, J = 18.2 Hz), 4.99 (1H, d, J = 7.6 Hz), 5.00 (2H, d, J = 18.2 Hz), 7.05 (1H, d, J = 8.0 Hz), 7.24–7.63 (4H, m), 7.50 (2H, d, J = 8.8 Hz), 7.68 (2H, d, J = 8.8 Hz), 9.64 (1H, s). Anal. calcd for $C_{27}H_{25}N_5O_5 \cdot 0.4H_2O$:C, 64.00; H, 5.13; N, 13.82. Found: C, 64.07; H, 5.17;N, 13.65.

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(tetrazol-4-ylmethylthio)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11xvii).

(1) 3-(Tetrazo-5-yl)methylaniline-trifluoroacetate. The compound was prepared by treating *m*-aminobenzenethiol in a similar manner described for the preparation of 3-(tetrazo-5-yl)methoxyaniline. ^1H NMR (DMSO-d_6) δ 4.53 (2H, s), 6.72–7.02 (3H, m), 7.10–7.28 (1H, m).

(2) **11xvii**. Mp 214–220 °C. ^{1}H NMR (DMSO-d_6) δ 0.79–1.02 (8H, m), 2.03–2.23 (2H, m), 4.33 (2H, s), 4.83 (2H, d, J = 18.0 Hz), 4.99 (1H, d, J = 8.0 Hz), 5.00 (2H, d, J = 18.0 Hz), 6.87–7.00 (2H, m), 7.00–7.18 (2H, m), 7.27–7.46 (5H, m), 9.18 (1H, s).

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(carboxymethylsulfoxy)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11xviii). To a solution of **11ix** (318 mg, 0.56 mmol) in CH_2Cl_2 (12 mL) and methanol (3 mL) was added 80% *m*-chloroperbenzoic acid (122 mg, 0.56 mmol) at −5 °C and the mixture was stirred for another 1 h. A 5% aq sodium thiosulfate solution was added to the mixture and the mixture was extracted with CH_2Cl_2. The organic layer was treated as usual. The crystalline residue was recrystallized from CH_2Cl_2–ether to obtain **11xviii** (140 mg; yield, 43%) as colorless crystals, mp 216–217 °C. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.96–1.18 (8H, m), 1.94–2.10 (2H, m), 3.65 (1H, d, J = 14 Hz) 3.83 (1H, d, J = 14 Hz), 3.83 (1H, d, J = 14 Hz), 4.79 (2H, d.d, J = 18, 2.6 Hz), 4.95 (2H, d, J = 18 Hz) 7.22–7.36 (7H, m), 7.67 (1H,s).

1,5-Bis-(cyclopropylcarbonylmethyl)-3-(*N'*-(*m*-(carboxymethylsulfoxy)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (11xix). The S-oxide, **11xviii** (250 mg, 0.43 mmol) was treated with a solution of excess diazomethane in ether. The crude product was purified by chromatography in ethyl acetate:methanol (100:0.5) and crystallized from CH_2Cl_2–ether to give **17-xix** (200 mg; yield, 78%) as colorless crystals, mp 208–210 °C. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.95–1.18 (8H, m), 1.88–2.08 (2H,m), 3.68 (1H, d, J = 14 Hz), 3.71 (3H, S), 3.82 (1H, d, J = 14 Hz), 4.79 (2H, dd, J = 11.4, 6.6 Hz), 4.96 (2H, d, d, J =14.8, 3.2 Hz), 5.31 (1H, d, J = 7.4 Hz), 6.71 (1H, d, J = 7.4 Hz), 7.13–7.43 (6H, m), 7.52 (1H, d, J = 9.4 Hz), 7.62 (1H, s), 8.43(1H, s).

1,5-Bis-(pyrrolidinocarbonylmethyl)-3-(*N'*-(*m*-(carboethoxy)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)-dione (12i). ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, J = 6.8 Hz), 1.69–2.00 (8H, m), 3.23–3.52 (8H, m), 4.29 (2H, q, J = 6.8 Hz), 4.49 (2H, d, J = 17.2 Hz), 4.72 (2H, d, J = 17.2 Hz), 5.00 (1H, d, J = 8.0 Hz), 6.87 (1H, d, J = 8.0 Hz), 7.31–7.60 (7H, m), 8.07 (1H, s), 9.49 (1H, s). Anal. calcd for $C_{43}H_{53}N_7O_6 \cdot 0.5H_2O$: C, 65.46; H, 6.96; N, 12.43. Found: C, 65.40; H, 6.91; N, 12.44.

1,5-Bis-(pyrrolidinocarbonylmethyl)-3-(*N'*-(*m*-(tetrazol-5-ylphenyl)ureido)-1*H*-1,5-benzodiazepine-2,4(3*H*,5*H*)-dione (12iv). Mp 290–293 °C. IR ν_{max} (KBr): 3425, 1705, 1642, 1570, 1507, 1455 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.64–1,98 (8H, m), 3.23–3.38 (4H, m), 3.42 (4H, m) 4.51 (2H, d, J = 17.4 Hz), 4.72 (2H, d, J = 17.4 Hz), 5.02 (1H, d, J = 8.2 Hz), 6.81 (1H, d, J = 8.2 Hz), 7.19 (1H, d, J = 8.2 Hz), 7.35–7.61 (7H, m), 7.82 (1H, s), 9.18 (1H, s). Anal. calcd for $C_{21}H_{27}N_5O_4 \cdot 0.7H_2O$: C, 59.20; H, 6.72; N, 16.44. Found: C, 59.38; H, 6.46; N, 16.20.

1,5-Bis-(pyrrolidinocarbonylmethyl)-3-(*N'*-(*m*-(carbomethoxymethyl)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (12xii). IR ν_{max} (KBr): 3425, 1735, 1691, 1652 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.84 (4H, qui, J = 6.2 Hz), 1.97 (4H, qui, J = 6.2 Hz), 3.36–3.58 (10H, m), 3.67 (3H, s) 4.61 (4H, s), 5.24 (1H, d, J = 7.0 Hz), 6.44 (1H, d, J = 7.4 Hz), 6.87 (1H, dt, J = 1.3, 7.4 Hz), 7.13(1H, m), 7.20–7.30 (2H, m), 7.30 (2H, m), 7.39 (1H, s), 7.52 (2H, m). Anal. calcd for $C_{31}H_{36}N_6O_7 \cdot 0.9H_2O$: C, 59.97; H, 6.14; N, 13.54. Found: C, 59.96; H, 6.17; N, 13.54.

1,5-Bis-(pyrrolidinocarbonylmethyl)-3-(*N'*-(*m*-(carboxymethyl)phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (12xiii). Mp 228–230 °C. IR ν_{max} (KBr): 3435, 3389, 1705, 1639, 1566 cm^{-1}. ^{1}H NMR(CD_3OD) δ 1.81–2.09 (8H, m), 3.44 (4H, t, J = 6.7 Hz), 3.52 (2H, s) 3.56 (4H, m), 4.66 (2H, d, J = 16.8 Hz), 4.76 (2H, d, J = 16.8 Hz), 5.19 (1H, s), 6.89 (1H, d, J = 7.4 Hz), 7.17 (1H, d, J = 7.4, 9.0 Hz), 7.23–7.31 (2H, m), 7.41 (2H, m), 7.58 (2H, m). Anal. calcd for $C_{30}H_{34}N_6O_7 \cdot 0.7H_2O$: C, 59.73; H, 5.91; N, 13.93. Found: C, 59.60; H, 5.82; N, 13.97.

1,5-Bis-(cyclopropylmethyl)-3-(*N'*-(*m*-tetrazolylphenyl)-ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (13iv). Mp 228–230 °C. IR ν_{max} (KBr): 3380, 1688, 1654, 1600, 1575,1543, 1500, 1425 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 0.03–0.22 (4H, m), 0.22–0.39 (4H,m), 0.71–0.92 (2H, m), 3.67 (2H, dd, J = 15.8, 7.0 Hz), 4.17 (2H, dd, J = 15.8, 7.0 Hz), 4.83 (1H, d, J = 7.8 Hz), 6.94 (1H, d, J = 7.8 Hz), 7.38–7.50 (4H, m), 7.52–7.59 (1H, m), 7.66–7.78 (2H, m), 8.15 (1H, s), 9.43 (1H, s). Anal. calcd for $C_{25}H_{26}N_8O_3 \cdot 1.7H_2O$: C, 58.06; H, 5.73; N, 21.6.7. Found: C, 58.30; H, 5.44; N, 21.35.

1,5-Bis-(2-thienylcarbonylmethyl)-3-(*N'*-(*m*-tetrazol-5-yl-phenyl)ureido)-1*H*-1,5-benzodiazepine-2,4-(3*H*,5*H*)dione (14iv). Mp 243–244 °C. IR ν_{max} (KBr): 3390, 1705, 1663, 1573, 1502,1408, 1240 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 5.15 (1H, d, J = 8.0 Hz), 5.30 (2H, d, J = 17.8 Hz), 5.55 (2H, d, J = 17.8 Hz), 6.93 (1H, d, J = 8.0 Hz), 7.27–7.60 (9H, m), 8.01 (1H, s), 8.06–8.20 (4H, m), 9.29 (1H, s). Anal. calcd for $C_{43}H_{53}N_7O_6 \cdot 0.5H_2O$: C, 65.46; H, 6.96; N, 12.43. Found: C, 65.40; H, 6.91; N, 12.44.

In vivo test for evaluation of inhibitory effect on gastric acid secretion by the schild method

Eight-week-old male Sprague–Dawly rats were starved for 24 h (ad libitum for water) and anesthetized with urethane (1.5 g kg^{-1}, sc). After tracheostomy, an esophagus cannula was inserted orally up to the proventriculus and ligated around the gastric cardiac. A perfusion cannula was inserted from the duodenum into the stomach and ligated around the pylorus. Another cannula was placed into the duodenum and ligated for drug administration. The stomach was perfused via the esophagus cannula with physiological saline (37 °C) while collecting the perfusate for a 15-min period. The perfusate was subjected to titration

with 0.01 N NaOH solution to determine the acidity. When the basal acid secretion became stable, pentagastrin (10 mg kg^{-1} h^{-1}) was administered in a sustained manner via the common carotid vein for about 90 min until the acid secretion reached approximately the highest level. The test compound (0.5% MC suspension) was then administered into the duodenum through the cannula. The perfusate was collected for a 15-min interval to monitor the acid secretion for 90 min. The percent inhibition was calculated as follows:

$$\text{Percent inhibition } (\%) = 100 \times (C - A)/(i - B)$$

where A is the minimum value of total acidity observed after the administration of the test compound; B is the total acidity immediately before pentagastrin administration; and C is the total acidity immediately before administration of the test compound.

In vitro test for evaluation of gastrin/CCK-B antagonism

The pharmacological effects of the compounds prepared above were evaluated in vitro with respect to antagonistic activity against the gastrin receptor, CCK-B receptor, or CCK-A receptor, using fundic glands of guinea pig, crude membrane specimens from a mouse cerebral cortex, or crude membrane specimens from a mouse pancreas, respectively. Male Hartley guinea pig (450–600 g) or male ddY mouse (24–30 g) were used.

Gastrin receptor antagonism

Male guinea pigs were killed by bleeding and the stomach was extracted from each animal immediately, from which the gastric glands were prepared.

Preparation of test compounds and procedures of the displacing assay

A 1 mM solution of the compound to be tested in DMSO was prepared and diluted with 50% DMSO to obtain a 10-fold dilution series. The reaction was initiated by the addition of gastric glands to solutions of different concentrations each containing ^{125}I-labeled gastrin (final concentration, 0.2 nM). The mixture was incubated for 30 min at 25 °C and centrifuged at 2000 rpm for 5 min, then the supernatant was removed by aspiration. To the pellet was added ice-cooled incubation buffer, followed by gentle mixing, immediate centrifugation, and removal of the supernatant by aspiration. The radioactivity was measured with a gamma counter. The same procedure was repeated using 50% DMSO solution or human gastrin I instead of a solution of test compound so as to obtain the control value pertaining to total binding or the value pertaining to nonspecific binding, respectively.

CCK-A receptor antagonism and CCK-B receptor antagonism

Male mice were killed by decapitation and the cerebral cortex (CCK-B) and pancreas (CCK-A) were extracted immediately. Each was mixed with 50 mM Tris–HCl buffer (pH 7.4) and homogenized with a Teflon glass homogenizer and polytron homogenizer to obtain crude membrane specimens.

Preparation of test compounds and procedures of the displacing assay

A 1 M solution of a compound to be tested in DMSO was prepared and diluted with 50% DMSO to obtain a 10-fold dilution series. The reaction was initiated by the addition of crude membrane specimens to solutions of different concentrations each containing [^{3}H]-CCK-8 (final concentration, 1 nM). The mixture was incubated for 90 min at 25 °C, filtered through a glass filter with aspiration and washed with a cooled 50 mM Tris buffer. After the addition of Aquazol-2 cocktail, the radioactivity was measured. The same procedure was repeated using 50% DMSO solution or Ceruletide instead of a solution of test compound, to obtain the control value pertaining to total binding or the value pertaining to nonspecific binding, respectively.

Calculation of IC_{50}

The IC_{50} was determined by plotting the ratio (%) of the specific binding of a test compound to that of the control on a semilogarithmic graph and obtaining the concentration corresponding to 50%: specific binding of control = total binding (cpm) − nonspecific binding (cpm), and specific binding of test compound = total binding (cpm) − nonspecific binding (cpm).

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