Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 7, pp 1411–1423, 1997

PII: S0968-0896(97)00075-8
0968-0896/97 $17.00 + 0.00

Synthesis and Structure–Activity Relationships of Dual Histamine H_2 and Gastrin Receptor Antagonists with Modified Benzodiazepine Skeletons

Yasuyuki Kawanishi,* Shoichi Ishihara, Tadahiko Tsushima,* Kaoru Seno, Sanji Hagishita, Michio Ishikawa and Yasunobu Ishihara

Shionogi Research Laboratories, Shionogi & Co., Ltd, Fukushima-ku, Osaka 553, Japan

Abstract—Three different types of dual histamine H_2 and gastrin receptor antagonists, e.g. those bearing a benzazepine, benzoxazepine, or benzothiazepine skeleton instead of the benzodiazepine one as a gastrin receptor antagonistic moiety were synthesized to reduce high hydrophobicity of parent compounds and evaluated for the dual activities. These skeletal modifications significantly potentiated the binding affinity of dual antagonists with histamine H_2 receptor but markedly diminished their binding affinity with the gastrin receptor and the gastrin versus CCK-A receptor selectivity. We evaluated in vivo gastric acid antisecretory activities for some representative compounds by the rat pylorus ligation method for 10 mg kg^{-1} dose by oral route. However, they exerted only low inhibitory activities for oral dose with % inhibition values ranging between 32 and 53%. © 1997 Elsevier Science Ltd.

Introduction

In order to study structure–activity relationships (SAR) of our previously reported dual histamine H_2 and gastrin receptor antagonists (dual H_2 and G-A),[1,2] e.g. **1**,[2] and also to improve their low oral absorbability, we have examined two types of chemical modifications to date—altering the binding mode of both histamine H_2 receptor antagonist (H_2A) and gastrin receptor antagonist (GA) pharmacophores from the normal head-to-tail manner to the reversed head-to-head one,[3] and decreasing to some extent the high molecular hydrophobicity of hybrid compounds,[4] the latter being the subject of the accompanying paper. However, both results had not been fruitful. This time we focused on the chemical modification of the benzodiazepine skeleton itself, as we suspected that the 3-urea-substituted benzodiazepine skeleton adopted as a prototype GA pharmacophore moiety might be responsible for the low oral absorbability of the dual antagonists. In fact, the representative GA L-365,260[5] itself displays significantly diminished oral absorbability compared to other well-known benzodiazepine drugs, particularly, for higher classes of animals.[6] We report here the synthesis and biological evaluation of three different types of dual H_2 and G-A which bear the benzazepine,[7,8] benzoxazepine,[9] or benzothiazepine[9] skeleton instead of the benzodiazepine skeleton as a GA moiety.

Chemistry

The benzazepine-type hybrid compounds **11a, b** were synthesized by coupling benzazepine carboxylic acid derivatives **10a, b** with the roxatidine amine derivative Rox-H. Their molecular structures and synthetic schemes are summarized in Scheme 1. The synthetic details are as follows.

The starting material **3** for the synthesis of benzazepine derivatives was prepared in several steps from α-tetralone **2** by a known method,[7,8] namely, the conversion to a seven-membered benzazepine skeleton by ring enlargement, chlorination with phosphorus pentachloride (PCl_5), reduction, azidation by sodium azide (NaN_3), *N*-alkylation with ethyl bromoacetate, and reduction of the azide group to the amino group by catalytic hydrogenation. Optical resolution of the resultant amine **3** with chiral dibenzoyl-L-tartaric acid

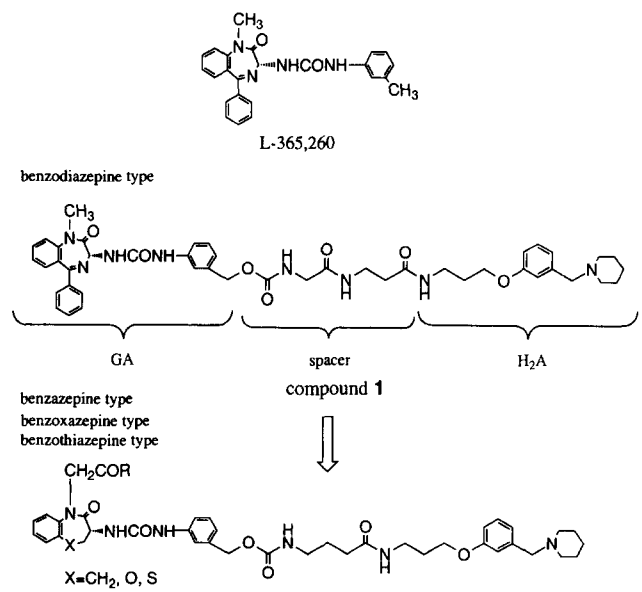
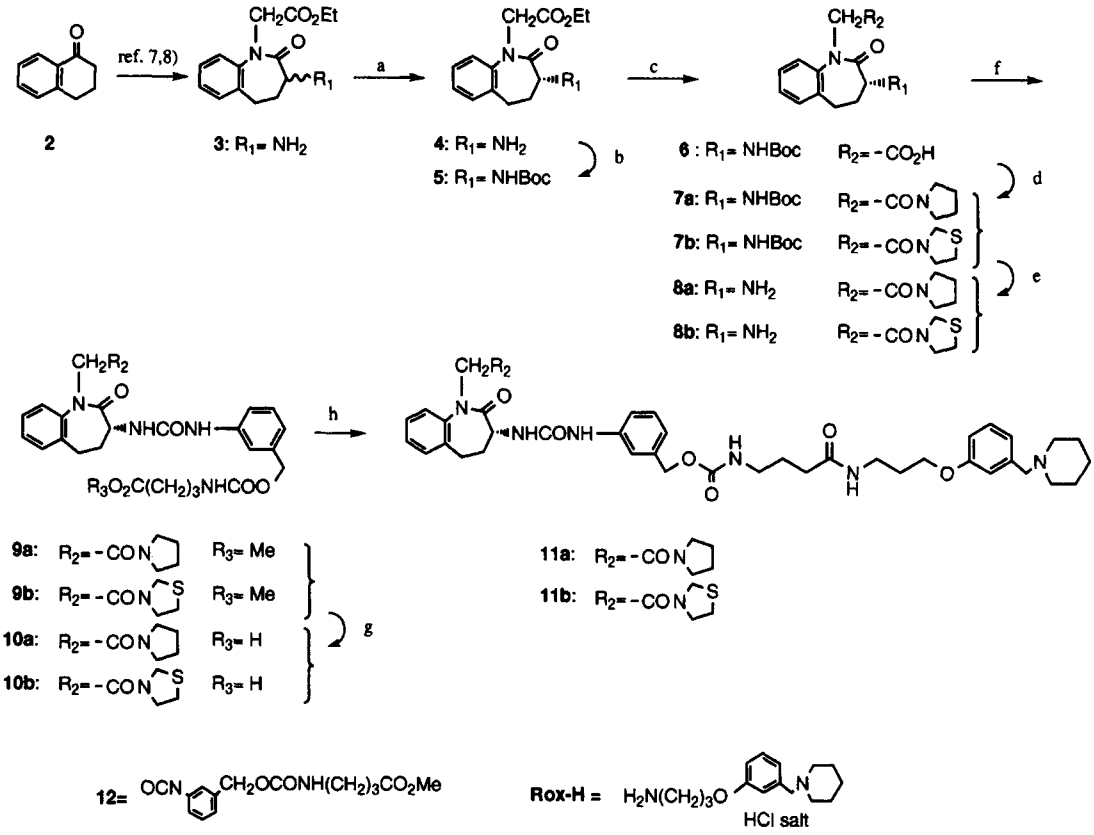


Figure 1.



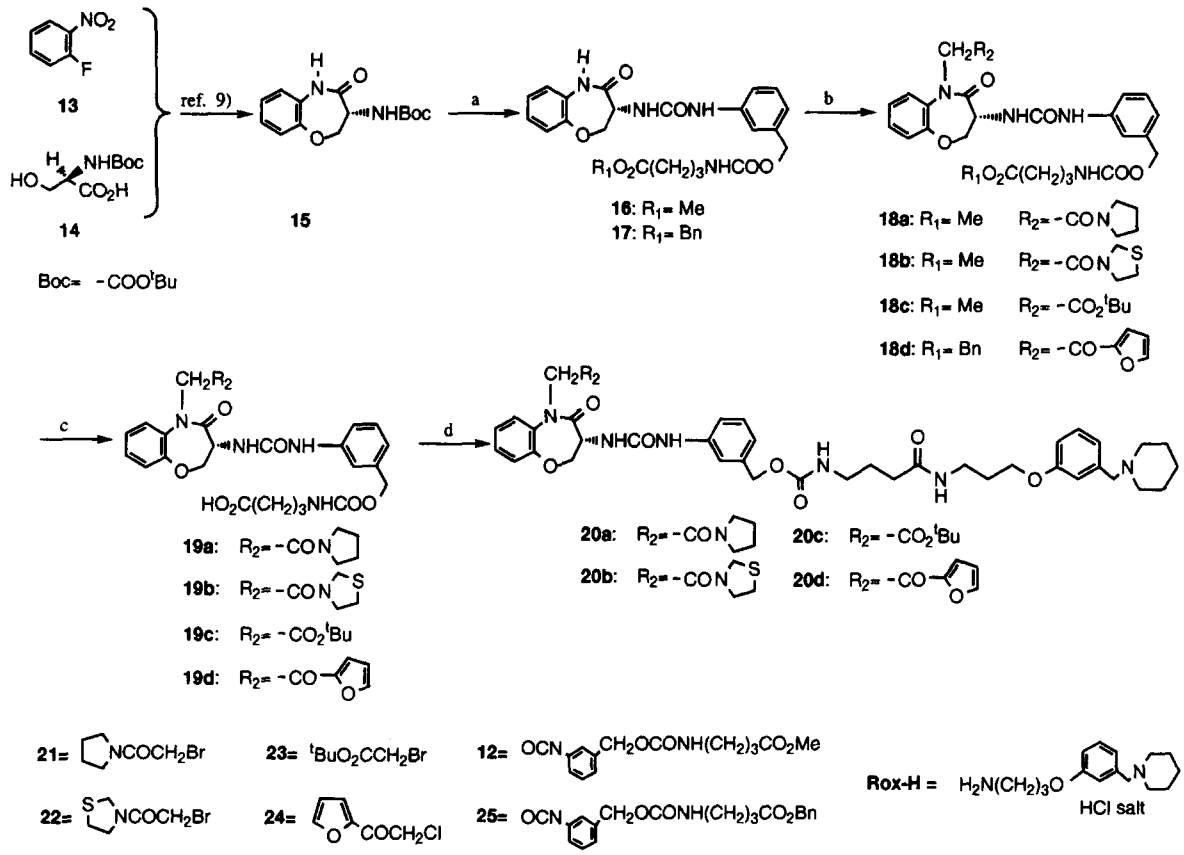
a: dibenzoyl-l-tartaric acid, resolution b: Boc_2O c: LiOH, aq.MeOH d: pyrrolidine or thiazolidine, HOBt, Et_3N, WSCI e: HCl / EtOAc
f: **12**, Et_3N g: LiOH, aq.MeOH h: **Rox-H**, HOBt, Et_3N, WSCI

Scheme 1.

gave optically pure (*R*) isomer **4**. After protecting the 3-amino functionality with *tert*-butylcarbamate (Boc), the resultant intermediate **5** was converted to carboxylic acid **6** by hydrolysis under basic conditions. The coupling reaction of **6** with either pyrrolidine and thiazolidine produced amides **7a** and **7b**, respectively. Deprotecting the amino group with 4 N hydrogen chloride (HCl)–EtOAc followed by condensation with isocyanate **12** in the presence of triethylamine (Et_3N) gave urea derivatives **9a, b** in the same way as previously reported.[4] Methyl esters **9a, b** were treated with aqueous lithium hydroxide (LiOH) to afford carboxylic acids **10a, b**. Finally, hybrid compounds **11a, b** were synthesized by coupling these benzazepine carboxylic acid derivatives **10a, b** with the roxatidine amine derivative Rox-H using water-soluble carbodiimide as a coupling agent.

The benzoxazepine-type hybrid compounds **20a, b, c,** and **d** were prepared by coupling the corresponding benzoxazepine carboxylic acid derivative **19a, b, c,** and **d** with the amine derivative of roxatidine Rox-H in almost the same way as described above. The molecular structures and synthetic schemes of these benzoxazepine-type hybrid compounds are summarized in Scheme 2. The details of their synthesis are as follows.

The key intermediate **15** for the modification of benzoxazepine derivatives was first prepared from 2-fluoronitrobenzene **13** and *N*-Boc-D-serine **14** in three steps by a reported procedure,[9] namely, *O*-phenylation of the serine hydroxyl group with 2-fluoronitrobenzene **13** in the presence of sodium hydride (NaH), catalytic reduction of the nitro group, and intramolecular cyclization to form the seven-membered benzoxazepine skeleton with diethyl phosphorocyanidate (DEPC). After deprotecting the C_3-amino functionality by treatment with 4 N HCl–EtOAc, the resultant free amino derivative was treated with isocyanates **12** and **25**, to yield methyl and benzyl ester, **16** and **17**, respectively. *N*-Alkylation of **16** and **17** with various bromides **21, 22, 23** and chloride **24** in the presence of powdered potassium hydroxide (KOH) and tetrabutylammonium bromide (TBAB) produced methyl esters **18a, b, c** and benzyl ester **18d** in the same way as previously reported.[4] These methyl and benzyl esters were converted into free carboxylic acid derivatives **19a–d** by alkaline hydrolysis in aqueous LiOH and catalytic hydrogenation using palladium (Pd)-charcoal as a catalyst, respectively. Finally, hybrid compounds were prepared by coupling these benzoxazepine carboxylic acid derivatives **19a–d** with the amine derivative of roxatidine Rox-H in the same way as described above.



a: 1) HCl / EtOAc 2) **12**, Et_3N or **25**, Et_3N b: **21** or **22** or **23** or **24**, KOH, TBAB c: LiOH, aq.MeOH, or Pd-C / H_2 d: **Rox-H**, HOBt, Et_3N, WSCI

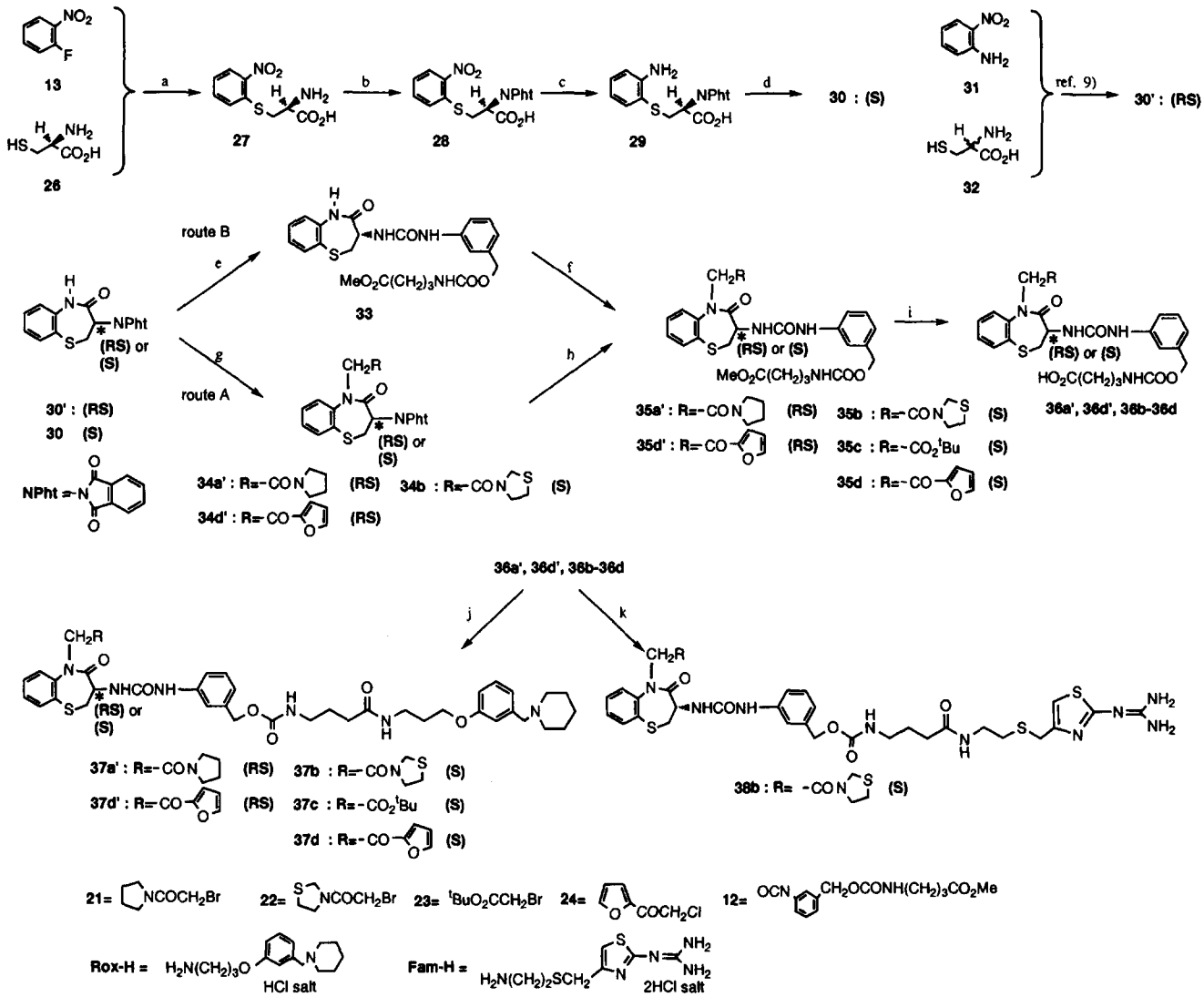
Scheme 2.

The benzothiazepine-type hybrid compounds **37a′**, **d′**, **b**, **c**, **d**, and **38b** were prepared by coupling benzothiazepine carboxylic acid derivatives **36a′**, **d′**, **b**, **c**, and **d** with the roxatidine or famotidine amine derivative Rox-H and Fam-H in the same way as described for benzazepine and benzoxazepine. The molecular structures and synthetic schemes of these benzothiazepine-type hybrid compounds are summarized in Scheme 3. Their synthetic details are as follows.

The racemic key intermediate **30′** for the synthesis of benzothiazepine derivatives could be prepared from 2-nitroaniline **31** and DL-cysteine **32** in four steps[9] involving diazotization of **31** and *S*-phenylation with **32** to give **30′**. However, as this procedure was quite impractical for scale-up production, we circumvented this process by nucleophilic aromatic substitution of 2-fluoronitrobenzene **13** with D-cysteine **26** in the presence of potassium *tert*-butoxide under a nitrogen atmosphere to obtain the *S*-arylated compound **27**. It was subsequently converted to **30**, the *S*-enantiomer of **30′**, in three steps involving *N*-protection with the phthalimide group, catalytic hydrogenation of the nitro group, and intramolecular cyclization with DEPC. Both intermediates **30** and **30′** were converted to methyl esters **35a′**, **d′**, **b**, **c**, and **d** by either route A or B in the following manner. Route A involved a sequence of reactions, *N*-alkylation of benzothiazepine, deprotection of their amino groups by treatment with hydrazine, and condensation with isocyanate **12**. This method gave the desired urea derivatives **35a′**, **d′**, **b**. Route B involved three sequences of reactions, deprotection of the amino group by treatment with hydrazine, condensation of the resultant free amino derivative with isocyanate **12**, and *N*-alkylation of **33** with ester **23** and halomethyl ketone **24**. This procedure gave the other desired *N*-alkylated urea compounds **35c** and **35d**. Route B appeared to be more practical than route A in producing various kinds of *N*-alkylated derivatives. The ester hydrolysis of these urea derivatives **35a′**, **d′**, **b**, **c** and **d** gave carboxylic acids **36a′**, **d′**, **b**, **c** and **d**, respectively. Finally, coupling the resultant benzothiazepine carboxylic acid derivatives **36a′**, **d′**, **b**, **c** and **d** with either the roxatidine amine derivative Rox-H or the famotidine derivative Fam-H as described above afforded the hybrid compounds listed in Table 1.

Biological Results and Discussion

The in vitro biological activities,[10,11,12] calculated hydrophobic parameters *Clog*P values, and chemical structures of the hybrid compounds and some other relevant compounds are summarized in Table 1. The *Clog*P



a: ^{t}BuOK, aq. MeOH / N_2 b: EtOOCNPht, Na_2CO_3 c: Pd-C / H_2 d: NCPO(OEt)$_2$ e: 1) hydrazine monohydrate 2) **12**, Et_3N f: **23** or **24**, KOH, TBAB g: **21** or **22** or **24**, KOH, TBAB h: 1) hydrazine monohydrate 2) **12**, Et_3N i: KOH, aq.MeOH j: **Rox-H**, HOBt, Et_3N, WSCI k: **Fam-H**, HOBt, Et_3N, WSCI

Scheme 3.

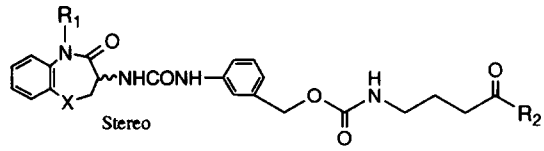
values were easily calculated using an appropriate computer program[13] as reported in our previous paper.[4]

The *Clog*P values in Table 1 showed a tendency for all these hybrid compounds to possess somewhat lower values (2.740–5.417) than that of the previously reported benzodiazepine compound **1** (5.740), indicating their increased molecular hydrophilicity. The increasing order of molecular hydrophilicity suggested for these three types of hybrid compounds is: benzazepine < benzothiazepine < benzoxazepine. The increasing order of hydrophilicity of *N*-substituents is: *tert*-butoxycarbonylmethyl < 2-oxo-2-thiazolidin-3-yl-ethyl < 2-furan-2-yl-2-oxo-ethyl < 2-oxo-2-pyrrolidin-1-yl-ethyl. Based on this theoretical background, we discuss here the SAR of the three types of hybrid compounds.

The most characteristic feature of the biological results obtained with these GA part-modified hybrid compounds is that their pA_2 values are greatly potentiated from those of previous benzodiazepine-type hybrid compounds, displaying values between 6.6 and 7.5 instead of values between 6.0 and 6.8, although a few exceptional compounds **20c** and **37c** exist. Surprisingly, compound **37b** displayed an extremely high pA_2 value of 7.5 which surpassed the 7.3 of the most potent H_2A of the marketed ones, namely, famotidine. The same pA_2 values of racemic and chiral compounds **37d'** and **37d** may suggest that the difference in the urea's configuration at the benzothiazepine C_3 position does not notably affect H_2A activities.

Unlike these H_2A activities, the GA activities of all these hybrid compounds markedly deteriorated to exhibit IC_{50} values between 88 and 680 nM. In addition, their GA versus CCK-A receptor selectivities were entirely lost with ratios less than 1.3 for the benzazepine and benzothiazepine types and slightly higher ratios,

Table 1. In vitro biological activities of hybrid compounds and derivatives



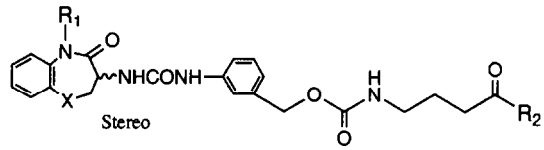
No.	X	R_1	R_2	Stereo	*ClogP*	Receptors IC_{50} (nM) Gastrin	CCK-B	CCK-A	Ratio Gastrin:CCK-A	pA_2 His.H_2
11a	CH_2	-CH_2CON	-Rox	R	4.534	240	>1000	320	1.3	6.6
11b	CH_2	-CH_2CON	-Rox	R	4.966	410	>1000	200	<1	6.6
20a	O	-CH_2CON	-Rox	R	3.980	100	>1000	1000	10.0	7.1
20b	O	-CH_2CON	-Rox	R	4.412	480	>1000	540	1.1	7.1
20c	O	-$CH_2CO_2{}^tBu$	-Rox	R	4.983	115	>1000	280	2.4	6.0
20d	O	-CH_2CO-	-Rox	R	4.233	88	480	360	4.1	6.9
37a′	S	-CH_2CON	-Rox	RS	4.414	680	>1000	760	1.1	7.0
37d′	S	-CH_2CO-	-Rox	RS	4.667	290	1150	190	<1	6.8
37b	S	-CH_2CON	-Rox	S	4.845	310	>1000	220	<1	7.5
37c	S	-$CH_2CO_2{}^tBu$	-Rox	S	5.417	195	>1000	160	<1	5.9
37d	S	-CH_2CO-	-Rox	S	4.667	250	380	<100	<1	6.9
38b	S	-CH_2CON	-Fam	S	2.740	135	560	<100	<1	6.9
1				R	5.740	19	103	8200	432	6.8
	L-365,260					4	29	11100	2775	
35a′	S	-CH_2CON	-OCH_3	RS		1350	>1000	1200	<1	
35d′	S	-CH_2CO-	-OCH_3	RS		290	>1000	<100	<1	
35b	S	-CH_2CON	-OCH_3	S		600	>1000	120	<1	
35c	S	-$CH_2CO_2{}^tBu$	-OCH_3	S		94	>1000	210	2.2	
		Cimetidine								6.6
		Roxatidine acetate								7.2
		Famotidine								7.3

Rox= —$NH(CH_2)_3O$— Fam= —$NH(CH_2)_2SCH_2$—

e.g. 10.0 and 4.1, for the benzoxazepine cases e.g. **20a** and **d**, respectively. The diminished GA activities and GA versus CCK-A receptor selectivities of hybrid compounds **37a′**, **d′**, **b** and **c** may correspond to the significantly diminished activities and selectivities of their nonhybridized ester precursors, **35a′**, **d′**, **b** and **c**, respectively.

Despite their relatively low GA activities, five representative compounds **11a**, **20a**, **20d**, **37b**, **38b** selected from three different types of GA part-modified hybrid compounds were evaluated for oral in vivo gastric acid antisecretory activities by the rat pylorus ligation method[14] at 10 mg kg^{-1} dose. However, all of them exhibited slightly lower inhibitory activity, ranging between 32 and 53%, than the magnitude 54% for compound **1**, suggesting no improvement in their oral absorbability (Table 2). Consequently, these findings seem to cast some doubts against our expectation that the oral absorbability of these hybrid compounds would be somewhat improved by decreasing their high hydrophobicity to a certain balanced level. In order to confirm here that the observed in vivo antigastric acid activity was displayed by the hybrid compounds themselves but not by their metabolites, we conducted brief stability tests of these hybrid compounds in artificial gastric or intestinal tract juices as well as in plasma membrane but any notable metabolic degrada-

Table 2. In vivo gastric acid antisecretory activity of hybrid compounds



No.	X	R_1	R_2	Stereo	*ClogP*	Inhibition (%)
11a	CH_2	$-CH_2CON$	-Rox	R	4.534	53
20a	O	$-CH_2CON$	-Rox	R	3.980	32
20d	O	$-CH_2CO-$	-Rox	R	4.233	40
37b	S	$-CH_2CON$	-Rox	S	4.845	39
38b	S	$-CH_2CON$	-Fam	S	2.740	42
1				R	5.740	54

Rox= $-NH(CH_2)_3O$ Fam= $-NH(CH_2)_2SCH_2$

tions were not observed. We therefore speculate that these in vivo dual antagonistic activities would be mainly associated with the hybrid compounds themselves but not their metabolites.

Conclusion

In this study, we synthesized three types of dual H_2 and G-A having a benzazepine, benzoxazepine, benzothiazepine skeleton as a different GA moiety from the previous benzodiazepine one. These skeletal modifications significantly potentiated the in vitro binding affinity with histamine H_2 receptor to the highest level. However, their in vivo oral gastric acid antisecretory activities measured by the rat pylorus ligation method at 10 mg kg^{-1} dose were only marginal with % inhibition values ranging between 32 and 53% and no significant improvement of their oral absorbability from compound **1** was observed. We are conducting other types of skeletal modifications to study SARs of dual H_2 and G-A and also to improve their oral absorbability.

Experimental

Chemistry

All melting points and softening points were determined on a Yanagimoto micromelting point apparatus and were not corrected. IR spectra were recorded on a Hitachi 260-10 IR spectrophotometer. ^1H NMR spectra were taken on a Varian VXR-200 spectrometer for organic solutions using tetramethylsilane (TMS) as an internal standard and their chemical shifts were given on a ppm scale. The optical rotations were measured on a Perkin–Elmer model 241 polarimeter. Column chromatography was performed on Merck Silica gel 60 (230–400 or 70–230 mesh).

Hybrid compounds

General procedure. 1-Hydroxybenzotriazole (HOBt) (103 mg, 0.76 mmol), triethylamine (Et_3N) (230 mg, 2.28 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSCI) (190 mg, 0.99 mmol) were added stepwise under ice cooling into a well-stirred solution containing the amine derivative of roxatidine Rox-H (247 mg, 0.99 mmol) and the benzazepine (or benzoxazepine or benzothiazepine) carboxylic acid derivative (0.76 mmol) in 5 ml of dimethylformamide (DMF). The reaction mixture was kept at room temperature for 16 h and concentrated under vacuum. The residue was extracted with chloroform ($CHCl_3$) and washed with H_2O, 5% sodium carbonate (Na_2CO_3), and H_2O, dried over magnesium sulfate ($MgSO_4$) and concentrated under vacuum. The residue was chromatographed on a silica gel column using $CHCl_3$–methanol (MeOH) (10:1–5:1, v/v) as an eluent to give the desired hybrid compound at 60–88% yield.

This method was used to prepare other hybrid compounds, except for compound **38b**.

(+)-(*R*)-{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]propyl}carbamic acid 3-{3-[2-oxo-1-(2-oxo-2-pyrrolidin-1-ylethyl)-2,3,4,5-tetrahydro-1H-benzo[b]azepin-3-yl]ureido}benzyl ester (11a). $[\alpha]^{23}_D$ +116.8 (*c* 0.978, MeOH). ^1H NMR ($CDCl_3$+CD_3OD) δ: 1.48 (br, 2H), 1.63 (br, 4H), 1.70–2.12 (m, 9H), 2.17–2.26 (m, 2H), 2.48 (br, 4H), 2.50–2.70 (m, 2H), 3.12–3.24 (m,

2H), 3.30–3.60 (m, 8H), 4.02 (br, 2H), 4.07 (d, 1H, J = 16.4 Hz), 4.42–4.54 (m, 1H), 4.76 (d, 1H, J = 15.6 Hz), 5.01 (s, 2H), 6.17 (d, 1H, J = 6.6 Hz), 6.70–7.00 (m, 4H), 7.10–7.40 (m, 8H). Anal. calcd for $C_{44}H_{57}N_7O_7 \cdot H_2O$: C, 64.92; H, 7.31; N, 12.05. Found: C, 64.86; H, 7.28; N, 12.20.

(+)-(*R*)-{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]propyl}carbamic acid 3-{3-[2-oxo-1-(2-oxo-2-thiazolidin-3-ylethyl)-2,3,4,5-tetrahydro-1H-benzo[b]azepin-3-yl]ureido}benzyl ester (11b). $[\alpha]^{24}_D$ +115.0 (*c* 1.077, MeOH). IR (KBr) cm^{-1}: 3354, 3076, 1701, 1653, 1600, 1556. ^1H NMR ($CDCl_3$) δ: 1.36–1.52 (m, 2H), 1.53–1.70 (m, 4H), 1.72–2.09 (m, 5H), 2.20 (t, 2H, J = 7.0 Hz), 2.38–2.72 (m, 6H), 2.80–3.00 (m, 1H), 3.06 (q, 1H, J = 6.2 Hz), 3.18 (q, 2H, J = 5.4 Hz), 3.33 (q, 2H, J = 6.2 Hz), 3.51 (s, 3H), 3.64–3.83 (m, 2H), 3.96 (t, 2H, J = 6.1 Hz), 4.36–4.60 (m, 4H), 4.74 (d, 1H, J = 16.2 Hz), 4.90 (s, 2H), 5.75 (br, 1H), 6.39 (d, 1H, J = 7.2 Hz), 6.72–7.35 (m, 13H), 7.78 (s, 1H). Anal. calcd for $C_{43}H_{55}N_7O_7S \cdot 1.4H_2O$: C, 61.54; H, 6.94; N, 11.68; S, 3.82. Found: C, 61.48; H, 6.69; N, 11.73; S, 3.77.

(+)-(*R*)-{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]propyl}carbamic acid 3-{3-[8-oxo-9-(2-oxo-2-pyrrolidin-1-ylethyl)-6,7,8,9-tetrahydro-5-oxo-9-azabenzocyclohepten-7-yl]ureido}benzyl ester (20a). $[\alpha]^{22}_D$ +123.3 (*c* 1.013, DMF). IR (nujol) cm^{-1}: 3317, 1651,1558, 1496. ^1H NMR ($CDCl_3$) δ: 1.48 (br, 2H), 1.64 (br, 4H), 1.75–2.10 (m, 6H), 2.21 (t, 2H, J = 7.2 Hz), 2.46 (br, 4H), 3.10–3.60 (m, 12H), 4.01 (t, 2H, J = 6.0 Hz), 4.16 (d, 1H, J = 16.8 Hz), 4.27 (d, 1H, J = 10.4 Hz), 4.67 (t, 1H, J = 8.6 Hz), 4.84 (d, 1H, J = 16.8 Hz), 4.92–4.99 (m, 1H), 5.00 (s, 2H), 6.70–7.00 (m, 4H), 7.10–7.40 (m, 8H). Anal. calcd for $C_{43}H_{55}N_7O_8 \cdot 0.8H_2O$: C, 63.58; H, 7.02; N, 12.07. Found: C, 63.56; H, 7.03; N, 12.16.

(+)-(*R*)-{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]propyl}carbamic acid 3-{3-[8-oxo-9-(2-oxo-2-thiazolidin-3-ylethyl)-6,7,8,9-tetrahydro-5-oxo-9-azabenzocyclohepten-7-yl]ureido}benzyl ester (20b). $[\alpha]^{22}_D$ +109.2 (*c* 1.212, MeOH). IR (nujol) cm^{-1}: 3321, 1658, 1558, 1496. ^1H NMR ($CDCl_3$+CD_3OD) δ: 1.47 (br, 2H), 1.64 (br, 4H), 1.74–1.88 (m, 2H), 1.96 (qui, 2H, J = 6.1 Hz), 2.22 (t, 2H, J = 7.3 Hz), 2.49 (br, 4H), 3.00 (t, 1H, J = 6.4 Hz), 3.14 (t, 2H, J = 6.6 Hz), 3.20 (br, 1H), 3.32–3.44 (m, 2H), 3.55 (br, 2H), 3.71–3.81 (m, 1H), 3.82–3.91 (m, 1H), 4.02 (t, 2H, J = 5.5 Hz), 4.05–4.40 (m, 2H), 4.50–4.80 (m, 3H), 4.85–5.10 (m, 2H), 5.00 (s, 2H), 6.80–7.10 (m, 5H), 7.10–7.40 (m, 7H). Anal. calcd for $C_{42}H_{53}N_7O_8S \cdot 0.5H_2O$: C, 61.15; H, 6.60; N,11.88; S, 3.89. Found: C, 61.13; H, 6.74; N, 11.82; S, 3.57.

(+)-(*R*)-{8-Oxo-7-[3-(3-{3-[3-(3-piperidin-1-ylmethylphenoxy)propylcarbamoyl]propylcarbamoyloxymethyl}-phenyl)ureido]-7,8-dihydro-6H-5-oxa-9-aza-benzocyclohepten-9-yl}acetic acid *tert*-butyl ester (20c). $[\alpha]^{22}_D$ +77.7 (*c* 1.025, MeOH). IR (nujol) cm^{-1}: 3321, 1721, 1672, 1559. ^1H NMR ($CDCl_3$+CD_3OD) δ: 1.44 (s, 9H), 1.46 (br, 2H), 1.55–1.70 (m, 4H), 1.81 (qui, 2H, J = 6.6 Hz), 1.96 (t, 2H, J = 6.3 Hz), 2.21 (t, 2H, J = 7.2 Hz), 2.49 (br, 4H), 3.10–3.25 (m, 2H), 3.30–3.45 (m, 2H), 3.54 (br, 2H), 4.02 (t, 2H, J = 6.0 Hz), 4.23 (dd, 1H, J = 9.8, 11.2 Hz), 4.29 (d, 1H, J = 17.0 Hz), 4.52 (d, 1H, J = 17.0 Hz), 4.64 (dd, 1H, J = 7.2, 9.4 Hz), 4.95 (dd, 1H, J = 7.4, 11.4 Hz), 6.80–7.10 (m, 5H), 7.10–7.40 (m, 7H). Anal. calcd for $C_{43}H_{56}N_6O_9 \cdot 1.7H_2O$: C, 62.11; H, 7.20; N, 10.11. Found: C, 62.08; H, 6.97; N, 10.16.

(+)-(*R*)-{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]propyl}carbamic acid 3-{3-[9-(2-furan-2-yl-2-oxoethyl)-8-oxo-6,7,8,9-tetrahydro-5-oxo-9-azabenzocyclohepten-7-yl]ureido}benzyl ester (20d). $[\alpha]^{24}_D$ +109.3 (*c* 1.011, $CHCl_3$). IR (nujol) cm^{-1}: 3290, 1660, 1553. ^1H NMR ($CDCl_3$+CD_3OD) δ: 1.43–1.59 (m, 2H), 1.60–1.84 (m, 6H), 1.86–2.03 (m, 2H), 2.22 (t, 2H, J = 6.8 Hz), 2.48–2.80 (m, 4H), 3.10–3.22 (m, 2H), 3.25–3.43 (m, 2H), 3.50–3.80 (m, 2H), 3.90–4.10 (m, 2H), 4.26 (t, 1H, J = 11.0 Hz), 4.66 (t, 1H, J = 7.4 Hz), 4.76 (t, 1H, J = 18.2 Hz), 5.00 (s, 2H), 5.90–5.12 (m, 1H), 5.41 (d, 1H, J = 18.0 Hz), 6.60–6.63 (m, 1H), 6.83–6.91 (m, 3H), 7.05 (s, 1H), 7.18–7.35 (m, 9H), 7.66 (s, 1H). Anal. calcd for $C_{43}H_{50}N_6O_9 \cdot 2.0H_2O$: C, 62.15; H, 6.55; N, 10.12. Found: C, 62.39; H, 6.42; N, 10.34.

{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-propyl}carbamic acid 3-{3-[4-oxo-5-(2-oxo-2-pyrrolidin-1-ylethyl)-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]ureido}benzyl ester (37a′). Softening point 93–95 °C. ^1H NMR ($CDCl_3$) δ: 1.36–1.66 (m, 6H), 1.68–1.98 (m, 8H), 2.12–2.26 (m, 2H), 2.38–2.50 (m, 4H), 3.02–3.50 (m, 9H), 3.48 (s, 2H), 3.70–3.81 (m, 1H), 3.88–3.98 (m, 3H), 4.69–4.78 (m, 1H), 4.83–5.08 (m, 3H), 6.61–7.65 (m, 12H). Anal. calcd for $C_{43}H_{55}N_7O_7S$: C, 63.44; H, 6.81; N, 12.05; S, 3.94. Found: C, 63.18; H, 6.71; N, 12.21; S, 3.88.

{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-propyl}carbamic acid 3-{3-[5-(2-furan-2-yl-2-oxo-ethyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]-ureido}benzyl ester (37d′). Softening point 88–90 °C. ^1H NMR ($CDCl_3$) δ: 1.35–1.70 (m, 6H), 1.74–2.02 (m, 4H), 2.18 (t, 2H, J = 5 Hz), 2.36–2.56 (m, 4H), 2.76–2.90 (m, 1H), 3.07–3.26 (m, 2H), 3.30–3.42 (m, 2H), 3.49 (s, 2H), 3.65–3.88 (m, 1H), 3.90–4.08 (m, 2H), 4.51, 5.55 (ABq, 2H, J = 18 Hz, Δν = 209.4 Hz), 4.70–4.86 (m, 1H), 4.94 (s, 2H), 6.50–6.55 (m, 1H), 6.72–7.74 (m, 14H). Anal. calcd for $C_{43}H_{50}N_6O_8S \cdot 2.2H_2O$: C, 60.71; H, 6.45; N, 9.88; S, 3.77. Found: C, 60.60; H, 6.24; N, 9.89; S, 3.46.

(+)-(*S*)-{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]propyl}carbamic acid 3-{3-[4-oxo-5-(2-oxo-2-thiazolidin-3-ylethyl)-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]ureido}benzyl ester (37b). Softening point 93–95 °C. $[\alpha]^{25}_D$ +125.9 (*c* 0.909, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 1.36–1.66 (m, 6H), 1.73–1.95 (m, 4H), 2.11–2.22 (m, 2H), 2.31–2.49 (m, 4H), 2.71–3.40 (m, 9H), 3.46 (s, 2H), 3.64–3.84 (m, 2H), 3.94 (t, 2H, J = 6.4 Hz), 4.41–5.12 (m, 6H), 6.72–7.81 (m, 12H). Anal. calcd for $C_{42}H_{55}N_7O_7S_2$: C, 60.48; H, 6.65; N, 11.76; S, 7.69. Found: C, 60.41; H, 6.39; N, 11.63; S, 7.58.

(+)-(*S*)-(4-Oxo-3-{3-[3-({3-piperidin-1-ylmethylphenoxy)-propylcarbamoyl]propyl}carbamoyloxymethyl)phenyl]-ureido}-3,4-dihydro-2H-1,5-benzothiazepin-5-yl)acetic acid *tert*-butyl ester (37c). ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9H), 1.35–2.01 (m, 10H), 2.13–2.27 (m, 2H), 2.42–2.63 (m, 4H), 2.86–3.03 (m, 1H), 3.15–3.30 (m, 2H), 3.30–3.46 (m, 2H), 3.55 (s, 2H), 3.74–3.85 (m, 1H), 4.00 (s, 2H), 4.12, 4.71 (ABq, 2H, J = 17.2 Hz, Δν = 118.4 Hz), 4.68–4.79 (m, 1H), 4.97 (s, 2H), 6.74–7.73 (m, 12H). Anal. calcd for $C_{43}H_{56}N_6O_8S{\cdot}0.4H_2O$: C, 62.66; H, 6.95; N, 10.20; S, 3.89. Found: C, 62.51; H, 6.98; N, 10.08; S, 3.82.

(*S*)-{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]propyl}carbamic acid 3-{3-[5-(2-furan-2-yl-2-oxoethyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]-ureido}benzyl ester (37d). ^{1}H NMR (CD_3OD) δ: 1.30–1.70 (m, 6H), 1.75–2.03 (m, 4H), 2.20 (t, 2H, J = 5 Hz), 2.35–2.58 (m, 4H), 2.81–3.02 (m, 1H), 3.06–3.25 (m, 2H), 3.30–3.42 (m, 2H), 3.48 (s, 2H), 3.65–3.78 (m, 1H), 3.90–4.06 (m, 2H), 4.51–5.62 (m, 5H), 6.65–7.88 (m, 15H). Anal. calcd for $C_{43}H_{50}N_6O_8S{\cdot}0.75H_2O$: C, 62.64; H, 6.30; N, 10.20; S, 3.89. Found: C, 62.90; H, 6.44; N, 10.34; S, 3.68.

(*S*)-{3-[2-(2-Guanidinothiazol-4-ylmethylthio)ethylcarbamoyl]propyl}carbamic acid 3-{3-[4-oxo-5-(2-oxo-2-thiazolidin-3-ylethyl)-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]ureido}benzyl ester (38b). HOBt (103 mg, 0.76 mmol), Et_3N (307 mg, 3.04 mmol), and WSCI (190 mg, 0.99 mmol) were added stepwise under ice cooling into a well-stirred solution containing the amine derivative of famotidine Fam-H (302 mg, 0.99 mmol) and benzothiazepine carboxylic acid derivative **36b** (445 mg, 0.76 mmol) in 5 ml of DMF. The reaction mixture was kept at room temperature for 16 h and concentrated under vacuum. The residue was thoroughly washed with cold water at least three times until the residue was well solidified, washed twice with cold 0.1 N sodium hydroxide (NaOH) solution and once with cold water, and then well dried under vacuum. Column chromatography of the residue on a silica gel column using a solvent mixture $CHCl_3$–MeOH (5:1, v/v) as an eluent gave **38b** (467 mg, 77%). Softening point 88–90 °C. ^{1}H NMR (CD_3OD) δ: 1.32 (t, 2H, J = 6 Hz), 1.69–1.83 (m, 2H), 2.18 (t, 2H, J = 7.4 Hz), 2.58 (t, 2H, J = 7 Hz), 2.85–3.22 (m, 7H), 3.64 (s, 2H), 3.67–3.86 (m, 2H), 4.27 (q, 1H, J = 8 Hz), 4.62–4.67 (m, 1H), 4.98 (s, 2H), 6.55 (s, 1H), 6.89–7.69 (m, 8H). Anal. calcd for $C_{34}H_{42}N_{10}O_6S_4{\cdot}H_2O$: C, 49.02; H, 5.32; N, 16.82; S, 15.40. Found: C, 49.21; H, 5.17; N, 17.02; S, 15.58.

Preparation of intermediates used for the synthesis of hybrid compounds

(+)-(*R*)-(3-Amino-2-oxo-2,3,4,5-tetrahydro-benzo[b]-azepin-1-yl)acetic acid ethyl ester (4). A solution of dibenzoyl-L-tartaric acid (9.16 g) in ethyl acetate (EtOAc) (50 ml) was added to a solution of **3** (6.30 g) in EtOAc (50 ml). The solution was allowed to stand for 16 h. The crystals were collected by filtration and recrystallized from EtOAc four times to give the salt (5.87 g). $[\alpha]^{24}_D$ +42.9 (*c* 1.141, MeOH).

The salt was shaken with aqueous sodium bicarbonate ($NaHCO_3$) and extracted with EtOAc. The extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from EtOAc:hexane to give **4** (1.88 g, 60%). Mp 104–105 °C. $[\alpha]^{24}_D$ +293.9 (*c* 1.164, EtOH). IR (nujol) cm^{-1}: 3360, 3310, 1744, 1660, 1190. ^{1}H NMR ($CDCl_3$) δ: 1.27 (t, 3H, J = 7.1 Hz), 1.92 (dt, 1H, J = 11.8, 7.4 Hz), 2.30–2.70 (m, 2H), 3.25 (dt, 1H, J = 13.2, 7.6 Hz), 3.35–3.55 (m, 1H), 4.20 (q, 2H, J = 7.0 Hz), 4.45 (d, 1H, J = 17.2 Hz), 4.64 (d, 1H, J = 17.2 Hz), 7.10–7.40 (m, 4H). Anal. calcd for $C_{14}H_{18}N_2O_3$:C, 64.10; H, 6.92; N, 10.68. Found: C, 63.95; H, 6.95; N, 10.54.

(+)-(*R*)-(3-*tert*-Butoxycarbonylamino-2-oxo-2,3,4,5-tetrahydrobenzo[b]azepin-1-yl)acetic acid ethyl ester (5). Protection of the amino group was performed by a common method using di-*tert*-butyl dicarbonate (Boc_2O) in a MeOH system. $[\alpha]^{25}_D$ +208.0 (*c* 0.854, MeOH). ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, 3H, J = 7.0 Hz), 1.39 (s, 9H), 1.97 (dd, 1H, J = 8.2, 11.4 Hz), 2.48–2.71 (m, 2H), 3.34 (dd, 1H, J = 7.8, 15.6 Hz), 4.10–4.37 (m, 3H), 4.35 (d, 1H, J = 17.2 Hz), 4.75 (d, 1H, J = 17.2 Hz), 5.43 (d, 1H, J = 7.8 Hz), 7.09–7.33 (m, 4H).

(*R*)-(3-*tert*-Butoxycarbonylamino-2-oxo-2,3,4,5-tetrahydrobenzo[b]azepin-1-yl)acetic acid (6). Compound **6** was prepared by the method used for compound **10a**. mp 147–148 °C (Et_2O–hexane). ^{1}H NMR ($CDCl_3$) δ: 1.38 (s, 9H), 1.97 (dd, 1H, J = 8.0, 11.1 Hz), 2.46–2.69 (m, 2H), 3.25 (dd, 1H, J = 7.8, 15.7 Hz), 4.20–4.35 (m, 1H), 4.43 (d, 1H, J = 17.6 Hz), 4.73 (d, 1H, J = 17.6 Hz), 5.47 (d, 1H, J = 8.2 Hz), 5.98 (br, 2H), 7.10–7.34 (m, 4H).

(*R*)-[2-Oxo-1-(2-oxo-2-pyrrolidin-1-ylethyl)-2,3,4,5-tetrahydro-1H-benzo[b]azepin-3-yl]carbamic acid *tert*-butyl ester (7a). Compound **7a** was prepared by the general procedure used for hybrid compounds. ^{1}H NMR ($CDCl_3$) δ: 1.39 (s, 9H), 1.77–2.08 (m, 5H), 2.48–2.71 (m, 2H), 3.37–3.67 (m, 5H), 4.21–4.37 (m, 1H), 4.37 (d, 1H, J = 16.0 Hz), 4.71 (d, 1H, J = 16.0 Hz), 5.45 (d, 1H, J = 7.6 Hz), 7.19 (m, 4H).

(+)-(*R*)-[2-Oxo-1-(2-oxo-2-thiazolidin-3-ylethyl)-2,3,4,5-tetrahydro-1H-benzo[b]azepin-3-yl]carbamic acid *tert*-butyl ester (7b). Compound **7b** was prepared by the general procedure used for hybrid compounds. $[\alpha]^{24}_D$ +239.2 (*c* 0.696, MeOH). IR ($CHCl_3$) cm^{-1}: 3429, 1707, 1660, 1602. ^{1}H NMR ($CDCl_3$) δ: 1.39 (s, 9H), 1.97(dd, 1H, J = 8.2, 11.3 Hz), 2.49–2.70 (m, 2H), 2.99 (t, 1H, J = 6.1 Hz), 3.14 (t, 1H, J = 6.1 Hz), 3.39–3.61 (m, 1H), 3.69–3.95 (m, 2H), 4.23–4.67 (m, 4H), 4.77 (d, 1H, J = 16.2 Hz), 5.41 (d, 1H, J = 7.6 Hz), 7.20 (m, 4H).

(*R*)-3-Amino-1-(2-oxo-2-pyrrolidin-1-ylethyl)-1,3,4,5-tetrahydrobenzo[b]azepin-2-one (8a). Deprotection of the amino group was performed by a common method using 4 N hydrochloric acid (HCl)–EtOAc in an EtOAc system and HCl salt was treated with $NaHCO_3$. ^{1}H

NMR ($CDCl_3$) δ: 1.80–2.10 (m, 4H), 2.30–2.70 (m, 3H), 3.25–3.39 (m, 1H), 3.40–3.60 (m, 4H), 4.44 (d, 1H, J = 16.2 Hz), 4.59 (d, 1H, J = 16.0 Hz), 7.10–7.30 (m, 4H).

(+)-(*R*)-3-Amino-1-(2-oxo-2-thiazolidin-3-ylethyl)-1,3,4,5-tetrahydrobenzo[b]azepin-2-one (8b). Compound **8b** was prepared by the same method used for compound **8a**. $[\alpha]^{24}_D$ +282.8 (*c* 0.993, MeOH). IR ($CHCl_3$) cm^{-1}: 3381, 3315, 1652, 1602. ^1H NMR ($CDCl_3$) δ: 1.80–2.15 (m, 3H), 2.43 (t, 1H, J = 7.4, 12.9 Hz), 2.60 (dd, 1H, J = 6.9, 13.3 Hz), 3.00 (t, 1H, J = 6.3 Hz), 3.15 (t, 1H, J = 6.3 Hz), 3.31 (dt, 1H, J = 7.8, 13.1 Hz), 3.48 (dd, 1H, J = 7.7, 11.5 Hz), 3.77–3.95 (m, 2H), 4.43–4.71 (m, 4H), 7.12–7.32 (m, 4H).

(*R*)-4-(3-{3-[2-Oxo-1-(2-oxo-2-pyrrolidin-1-ylethyl)-2,3,4,5-tetrahydro-1H-benzo[b]azepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid methyl ester (9a). Compound **9a** was prepared by the method used for compound **16**. Mp 183–184 °C (EtOAc). IR ($CDCl_3$) cm^{-1}: 3310, 1730, 1693, 1648, 1635, 1523, 1245. ^1H NMR ($CDCl_3$) δ: 1.70–1.90 (m, 4H), 1.90–2.00 (m, 2H), 2.33 (t, 2H, J = 7.5 Hz), 2.30–2.70 (m, 3H), 3.10–3.25 (m, 2H), 3.35–3.70 (m, 5H), 3.65 (s, 3H), 4.22 (d, 1H, J = 16.4 Hz), 4.49 (t, 1H, J = 9.6 Hz), 4.86 (d, 1H, J = 16.0 Hz), 4.95 (s, 2H), 6.88 (d, 1H, J = 7.4 Hz), 7.10–7.40 (m, 7H). Anal. calcd for $C_{30}H_{37}N_5O_7$: C, 62.16; H, 6.43; N, 12.08. Found: C, 61.98; H, 6.51; N, 11.99.

(+)-(*R*)-4-(3-{3-[2-Oxo-1-(2-oxo-2-thiazolidin-3-ylethyl)-2,3,4,5-tetrahydro-1H-benzo[b]azepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid methyl ester (9b). Compound **9b** was prepared by the method used for compound **16**. $[\alpha]^{24}_D$ +160.6 (*c* 0.954, MeOH). IR ($CHCl_3$) cm^{-1}: 3433, 3353, 1722, 1651, 1613, 1601. ^1H NMR ($CDCl_3$) δ: 1.82 (qui, 2H, J = 7.2 Hz), 1.95–2.14 (m, 1H), 2.35 (t, 2H, J = 7.2 Hz), 2.45–2.72 (m, 2H), 2.92 (t, 1H, J = 5.9 Hz), 3.07 (t, 1H, J = 5.8 Hz), 3.19 (q, 2H, J = 6.5 Hz), 3.40–3.62 (m, 1H), 3.66 (s, 3H), 3.70–3.85 (m, 2H), 4.35–4.61 (m, 4H), 4.80 (d, 1H, J = 16.0 Hz), 4.89 (s, 2H), 5.36 (br, 1H), 6.33 (br, 1H), 6.84 (d, 1H, J = 7.0 Hz), 7.02–7.35 (m, 7H), 7.47 (s, 1H). Anal. calcd for $C_{29}H_{35}N_5O_7S$ $0.2H_2O$: C, 57.93; H, 5.96;N, 11.72; S, 5.16. Found: C, 57.91; H, 5.96; N, 11.72; S, 5.16.

(*R*)-4-(3-{3-[2-Oxo-1-(2-oxo-2-pyrrolidin-1-ylethyl)-2,3,4,5-tetrahydro-1H-benzo[b]azepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid (10a). A solution of lithium hydroxide (LiOH) (0.42 g) in H_2O (4 ml) was added to a suspension of **9a** (1.61 g) in MeOH (8 ml). The mixture was stirred at room temperature for 16 h. Water was added to the reaction mixture. The mixture was washed with EtOAc, acidified with 1 N HCl, and extracted with dichloromethane (CH_2Cl_2)–MeOH mixture. The extracts were washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. The residue (1.64 g) was used for the next step without further purification. ^1H NMR ($CDCl_3$+CD_3OD) δ: 1.75–2.20 (m, 7H), 2.34 (t, 2H, J = 7.3 Hz), 2.44–2.70 (m, 2H), 3.21 (t, 2H, J = 6.7 Hz), 3.36–3.59 (m, 5H), 4.34 (d, 1H, J = 16.2 Hz), 4.49 (dd, 1H, J = 7.6, 11.8 Hz), 5.01 (s, 2H), 6.88 (d, 1H, J = 7.2 Hz), 7.10–7.50 (m, 7H).

(+)-(*R*)-4-(3-{3-[2-Oxo-1-(2-oxo-2-thiazolidin-3-ylethyl)-2,3,4,5-tetrahydro-1H-benzo[b]azepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid (10b). Compound **10b** was prepared by the method used for compound **10a**. $[\alpha]^{24}_D$ +161.6 (*c* 1.245, MeOH). IR ($CHCl_3$) cm^{-1}: 3345, 3352, 1712, 1645, 1600. ^1H NMR ($CDCl_3$) δ: 1.82 (qui, 2H, J = 6.8 Hz), 1.94–2.14 (m, 1H), 2.34 (t, 2H, J = 7.0 Hz), 2.49–2.71 (m, 2H), 2.98 (t, 1H, J = 5.4 Hz), 3.14 (t, 1H, J = 5.8 Hz), 3.21 (t, 2H, J = 6.7 Hz), 3.35–3.65 (m, 1H), 3.65–3.90 (m, 2H), 4.33–4.63 (m, 4H), 4.82 (d, 1H, J = 16.2 Hz), 5.01 (s, 2H), 6.89 (d, 1H, J = 7.0 Hz), 7.11–7.37 (m, 7H). Anal. calcd for $C_{28}H_{33}N_5O_7S{\cdot}0.4H_2O$: C, 56.91; H, 5.77; N, 11.86; S, 5.43. Found: C, 57.04; H, 5.95; N, 11.75; S, 5.30.

(+)-(*R*)-4-{3-[3-(8-Oxo-6,7,8,9-tetrahydro-5-oxa-9-aza-benzocyclohepten-7-yl)ureido]benzyloxycarbonylamino}-butyric acid methyl ester (16). Deprotection of the amino group was performed by a common method using 4 N HCl–EtOAc in EtOAc system. A solution of **12** (484 mg) in CH_2Cl_2 (5 ml) was added to a mixture of amine HCl salt (355 mg) and Et_3N (1.5 ml) in CH_2Cl_2 (5 ml). The solution was allowed to stand for 16 h. Methanol was added to this clean solution. The solution was washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column using $CHCl_3$–MeOH (10:1, v/v) as an eluent to give **16** (723 mg, 93%). $[\alpha]^{22}_D$ +93.3 (*c* 1.028, MeOH). IR (nujol) cm^{-1}: 3308, 1736, 1689, 1672, 1641, 1547. ^1H NMR ($CDCl_3$+CD_3OD) δ: 1.82 (qui, 2H, J = 7.0 Hz), 2.37 (t, 2H, J = 7.4 Hz), 3.19 (t, 2H, J = 6.9 Hz), 3.67 (s, 3H), 4.25 (t, 1H, J = 10.4 Hz), 4.68 (dd, 1H, J = 6.6, 10.2 Hz), 4.88 (dd, 1H, J = 10.6, 4.0 Hz), 5.02 (s, 2H), 6.90–7.40 (m, 8H). Anal. calcd for $C_{23}H_{26}N_4O_7{\cdot}0.2H_2O$: C,58.27; H, 5.61; N, 11.82. Found: C, 58.29; H, 5.59; N, 11.87.

(+)-(*R*)-4-{3-[3-(8-Oxo-6,7,8,9-tetrahydro-5-oxa-9-aza-benzocyclohepten-7-yl)ureido]benzyloxycarbonylamino}-butyric acid benzyl ester (17). Compound **17** was prepared by a similar method to that used for compound **16**. $[\alpha]^{22}_D$ +82.6 (*c* 1.029, MeOH). IR (nujol) cm^{-1}: 3301, 1733, 1688, 1669, 1636, 1544. ^1H NMR ($CDCl_3$+CD_3OD) δ: 1.83 (qui, 2H, J = 7.2 Hz), 2.40 (t, 2H, J = 7.2 Hz), 3.17 (t, 2H, J = 6.9 Hz), 4.24 (t, 2H, J = 10.4 Hz), 4.68 (dd, 1H, J = 6.6, 9.8 Hz), 4.90 (dd, 1H, J = 6.6, 10.6 Hz), 5.02 (s, 2H), 5.10 (s, 2H), 6.90–7.40 (m, 13H). Anal. calcd for $C_{29}H_{30}N_4O_7$: C, 63.72; H, 5.53; N, 10.25. Found: C, 63.47; H, 5.72; N, 10.24.

(+)-(*R*)-4-(3-{3-[8-Oxo-9-(2-oxo-2-pyrrolidin-1-ylethyl)-6,7,8,9-tetrahydro-5-oxa-9-azabenzocyclohepten-7-yl]-ureido}benzyloxycarbonylamino)butyric acid methyl ester (18a). Compound **18a** was prepared by the method used for compound **34a′**. $[\alpha]^{22}_D$ +186.1 (*c* 1.004, $CHCl_3$). IR (nujol) cm^{-1}: 3339, 1728, 1651, 1556. ^1H NMR ($CDCl_3$) δ: 1.70–2.00 (m, 6H), 2.31 (t, 2H, J =

7.4 Hz), 3.13–3.23 (m, 2H), 3.34–3.58 (m, 4H), 3.65 (s, 3H), 4.11 (d, 1H, J = 16.4 Hz), 4.32 (t, 1H, J = 10.4 Hz), 4.65 (dd, 1H, J = 9.6, 7.6 Hz), 4.80–5.10 (m, 4H), 5.49 (t, 1H, J = 6.0 Hz), 6.87 (d, 1H, J = 6.8 Hz), 6.84 (d, 1H, J = 8.2 Hz), 7.00–7.40 (m, 7H), 7.74 (s, 1H). Anal. calcd for $C_{29}H_{35}N_5O_8$: C, 59.88; H, 6.07; N, 12.04. Found: C, 59.77; H, 6.17; N, 11.89.

(+)-(*R*)-4-(3-{3-[8-Oxo-9-(2-oxo-2-thiazolidin-3-ylethyl)-6,7,8,9-tetrahydro-5-oxa-9-azabenzocyclohepten-7-yl]-ureido}benzyloxycarbonylamino)butyric acid methyl ester (18b). Compound **18b** was prepared by the method used for compound **34a′**. $[\alpha]^{22}_D$ +180.4 (*c* 1.008, $CHCl_3$). IR (nujol) cm^{-1}: 3341, 1717, 1655, 1557. ^1H NMR ($CDCl_3$) δ: 1.82 (qui, 2H, J = 7.2 Hz), 2.32 (t, 2H, J = 7.2 Hz), 2.88–3.02 (m, 1H), 3.11 (t, 1H, J = 6.2 Hz), 3.20 (br, 2H), 3.66 (s, 3H), 3.70–3.90 (m, 2H), 4.20–4.40 (m, 2H), 4.50–4.80 (m, 3H), 4.80–5.10(m, 4H), 6.90 (d, 1H, J = 7.4 Hz), 7.10–7.40 (m, 3H). Anal. calcd for $C_{28}H_{33}N_5O_8S$: C, 56.08; H, 5.55;N, 11.68; S, 5.35. Found: C, 56.10; H, 5.64; N, 11.52; S, 5.12.

(+)-(R)-4-{3-[3-(9-tert-Butoxycarbonylmethyl-8-oxo-6,7,8,9-tetrahydro-5-oxa-9-azabenzocyclohepten-7-yl)-ureido]benzyloxycarbonylamino}butyric acid methyl ester (18c). Compound **18c** was prepared by the method used for compound **34a′**. $[\alpha]^{22}_D$ +103.6 (*c* 1.124, $CHCl_3$). IR (nujol) cm^{-1}: 3347, 1733, 1667, 1557. ^1H NMR ($CDCl_3$) δ: 1.44 (s, 9H), 1.82 (qui, 2H, J = 7.0 Hz), 2.36 (t, 2H, J = 7.3 Hz), 3.20 (t, 2H, J = 6.9 Hz), 3.66 (s, 3H), 4.25 (dd, 1H, J = 11.2, 10.0 Hz), 4.31 (d, 1H, J = 17.0 Hz), 4.54 (d, 1H, J = 17.2 Hz), 4.65 (dd, 1H, J = 7.4, 9.8 Hz), 4.97 (dd, 1H, J = 7.6, 11.4 Hz), 5.00 (s, 2H), 6.93 (d, 1H, J = 7.0 Hz), 7.10–7.40 (m, 7H). Anal. calcd for $C_{29}H_{36}N_4O_9$:C, 59.58; H, 6.21; N, 9.59. Found: C, 59.47; H, 6.25; N, 9.52.

(*R*)-4-(3-{3-[9-(2-Furan-2-yl-2-oxoethyl)-8-oxo-6,7,8,9-tetrahydro-5-oxa-9-azabenzocyclohepten-7-yl]ureido}-benzyloxycarbonylamino)butyric acid benzyl ester (18d). Compound **18d** was prepared by the method used for compound **34a′**. ^1H NMR ($CDCl_3$) δ: 1.82 (qui, 2H, J = 7.4 Hz), 2.38 (t, 2H, J = 7.6 Hz), 3.18 (q, 2H, J = 6.2 Hz), 4.26 (t, 1H, J = 10.2 Hz), 4.66 (t, 1H, J = 10.4 Hz), 4.70 (d, 1H, J = 18.0 Hz), 4.95 (s, 2H), 4.96-5.10 (m, 1H), 5.10 (s, 2H), 5.24 (t, 1H, J = 5.6 Hz), 5.43 (d, 1H, J = 17.6 Hz), 6.34 (d, 1H, J = 6.6 Hz), 6.53–6.56 (m, 1H), 6.89 (d, 1H, J = 7.0 Hz), 7.12–7.40 (m, 13H), 7.59 (d, 1H, J = 1.2 Hz). Anal. calcd for $C_{35}H_{34}N_4O_9$ H_2O: C, 62.49; H, 5.39; N, 8.33. Found: C, 62.46; H, 5.29; N, 8.30.

(*R*)-4-(3-{3-[8-Oxo-9-(2-oxo-2-pyrrolidin-1-ylethyl)-6,7,8,9-tetrahydro-5-oxa-9-azabenzocyclohepten-7-yl]ureido}-benzyloxycarbonylamino)butyric acid (19a). Compound **19a** was prepared by the method used for compound **10a**. IR (nujol) cm^{-1}: 3338, 1651, 1558. ^1H NMR ($CDCl_3$) δ: 1.61–1.86 (m, 4H), 1.88–2.04 (m, 2H), 2.20–2.36 (m, 2H), 3.05–3.23 (m, 2H), 3.33–3.59 (m, 4H), 4.05 (d, 1H, J = 16.0 Hz), 4.35 (t, 1H, J = 10.6 Hz), 4.63 (t, 1H, J = 8.6 Hz), 4.91 (s, 2H), 4.99 (s, 2H), 5.77 (br, 1H), 6.37–6.55 (m, 1H), 6.72–6.88 (m, 1H), 7.00–7.50 (m, 7H), 7.95 (s, 1H).

(*R*)-4-(3-{3-[8-Oxo-9-(2-oxo-2-thiazolidin-3-ylethyl)-6,7,8,9-tetrahydro-5-oxa-9-azabenzocyclohepten-7-yl]-ureido}benzyloxycarbonylamino)butyric acid (19b). Compound **19b** was prepared by the method used for compound **10a**. ^1H NMR ($CDCl_3$+CD_3OD) δ: 1.73–1.91 (m, 2H), 2.34 (t, 2H, J = 7.1 Hz), 3.01 (t, 2H, J = 6.4 Hz), 3.11–3.27 (m, 3H), 3.70–3.95 (m, 2H), 4.15–4.40 (m, 2H), 4.50–4.75 (m, 3H), 4.80–5.10 (m, 4H), 6.91 (d, 1H, J = 7.6 Hz).

(*R*)-4-{3-[3-(9-*tert*-Butoxycarbonylmethyl-8-oxo-6,7,8,9-tetrahydro-5-oxa-9-azabenzocyclohepten-7-yl)ureido]-benzyloxycarbonylamino}butyric acid (19c). Compound **19c** was prepared by the method used for compound **10a**. ^1H NMR ($CDCl_3$) δ: 1.42 (s, 9H), 1.72–1.92 (m, 2H), 2.35 (t, 2H, J = 6.5 Hz), 3.12–3.29 (m, 2H), 4.30 (d, 1H, J = 17.2 Hz), 4.31 (dd, 1H, J = 9.8, 9.0 Hz), 4.55 (d, 1H, J = 17.0 Hz), 4.63 (dd, 1H, J = 9.4, 7.0 Hz), 4.90–5.10 (m, 3H), 6.86 (d, 1H, J = 7.4 Hz), 7.10–7.50 (m, 7H).

(*R*)-4-(3-{3-[9-(2-Furan-2-yl-2-oxoethyl)-8-oxo-6,7,8,9-tetrahydro-5-oxa-9-azabenzocyclohepten-7-yl]ureido}-benzyloxycarbonylamino)butyric acid (19d). A mixture of **18d** (438 mg) and 10% palladium–charcoal (50 mg) in MeOH (10 ml) was stirred under hydrogen for 20 min. After ca. 18 ml of hydrogen was absorbed, the catalyst was filtered and washed with MeOH. The filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column using $CHCl_3$–MeOH (5:1, v/v) as an eluent to give **19d** (283 mg, 77%). ^1H NMR ($CDCl_3$+CD_3OD) δ: 1.81 (qui, 2H, J = 7.0 Hz), 2.34 (t, 2H, J = 7.4 Hz), 3.19 (t, 2H, J = 7.0 Hz), 4.27 (dd, 1H, J = 9.8, 11.0 Hz), 4.67 (dd, 1H, J = 7.4, 9.8 Hz), 4.85 (d, 1H, J = 18.0 Hz), 4.96–5.14 (m, 3H), 5.42 (d, 1H, J = 18.0 Hz), 6.63–6.66 (m, 1H), 6.92–7.02 (m, 1H), 7.18–7.47 (m, 8H), 7.71 (s, 1H).

Amino-(2-nitrophenylsulfanyl)acetic acid (27). To an ice-cooled solution of d-cysteine HCl H_2O (25 g, 142 mmol) in 200 ml of aq. MeOH (MeOH 120 ml, H_2O 80 ml) were added potassium *tert*-butoxide (^{t}BuOK) (52.7 g, 470 mmol) and 2-fluoronitrobenzene (20.1 g, 142 mmol) after the solution was substituted with nitrogen thoroughly. The reaction mixture was kept at the same temperature for 20 min and at room temperature for 2 h, then concentrated under vacuum. After acidification with 1 N sulfuric acid (H_2SO_4), the residue was concentrated to one-third of the volume under vacuum again. The pH of this residue was adjusted to 10 with 10% ammomia solution (NH_4OH). After the residue was again concentrated under vacuum, the deposited crystals were collected by filtration, suspended in ice-cooled water, collected by filtration again, washed with ice-cooled water and dried in vacuo. The first mother liquor was concentrated to obtain a crop of second crystals in the same way as for the first crystals. (Total 33 g, 96%.)

(*S*)-4-{3-[3-(4-Oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl)-ureido]benzyloxycarbonylamino}butyric acid methyl ester (33). Compound **33** was prepared by the method used for compound **35a′**. ^{1}H NMR (CD_3OD) δ: 1.71–1.85 (m, 2H), 2.33 (t, 2H, J = 7 Hz), 3.01 (t, 1H, J = 11 Hz), 3.10–3.19 (m, 2H), 3.64 (s, 3H), 3.71–3.83 (m, 1H), 4.50–4.62 (m, 1H), 4.99 (s, 2H), 6.91–7.02 (m, 1H), 7.15–7.50 (m, 6H), 7.62–7.71 (m, 1H).

2-[4-Oxo-5-(2-oxo-2-pyrrolidine-1-ylethyl)-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl]isoindole-1,3-dione (34a′). To an ice-cooled suspension of **30′** (100 mg, 0.31 mmol), powdered potassium hydroxide (KOH) (22 mg, 0.39 mmol), and tetrabutylammonium bromide (TBAB) (100 mg, 0.31 mmol) in 10 ml of tetrahydrofuran (THF) was added bromide **21** (77 mg, 0.40 mmol). The reaction mixture was kept at room temperature for 2 h. The precipitates were filtered off and the filtrate was concentrated in vacuo. The residue was extracted with $CHCl_3$ and washed with 1 N HCl, 5% Na_2CO_3 and H_2O, dried over $MgSO_4$ and concentrated under vacuum. The residue was chromatographed on a silica gel column using CH_2Cl_2–EtOAc (2:1, v/v) as an eluent to give **34a′** (97 mg, 72%) as a powder. ^{1}H NMR ($CDCl_3$) δ: 1.74–2.04 (m, 4H), 3.36–3.60 (m, 5H), 3.97, 4.96 (ABq, 2H, J = 16 Hz, Δν = 200 Hz), 4.57 (t, 1H, J = 11 Hz), 7.23–7.32 (m, 1H), 7.42–7.54 (m, 1H), 7.64–7.74 (m. 4H), 7.78–7.86 (m, 2H).

(*S*)-2-[4-Oxo-5-(2-oxo-2-thiazolidine-3-ylethyl)-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl]isoindole-1,3-dione (34b). Compound **34b** was prepared by the method used for compound **34a′**. ^{1}H NMR ($CDCl_3$) δ: 2.90–3.16 (m, 2H), 3.46–3.57 (m, 1H), 3.67–3.92 (m, 2H), 4.02–4.13 (m, 1H), 4.56, 4.97 (ABq, 2H, J = 16 Hz, Δν = 82.9 Hz), 4.44–4.66 (m, 1H), 4.58 (t, 1H), 5.11 (dd, 1H, J = 12.2, 7.6 Hz), 7.22–7.34 (m, 1H), 7.42–7.55 (m, 1H), 7.62–7.76 (m, 4H), 7.78–7.88 (m, 2H).

2-[5-(2-Furan-2-yl-2-oxoethyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-3-yl]isoindole-1,3-dione (34d′). Compound **34d′** was prepared by the method used for compound **34a′**. ^{1}H NMR ($CDCl_3$) δ: 3.54 (dd, 1H, J = 11.8, 7.4 Hz), 4.47, 5.59 (ABq, 2H, J = 17.6 Hz, Δν = 224 Hz), 4.61 (t, 1H, J = 11.8 Hz), 5.15 (dd, 1H, J = 11.8, 7.4 Hz), 6.48–6.57 (m, 1H), 7.20–7.86 (m, 10H).

4-(3-{3-[4-Oxo-5-(2-oxo-2-pyrrolidine-1-ylethyl)-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid metyl ester (35a′). The mixture of **34a′** (100 mg, 0.23 mmol) and hydrazine monohydrate (35 mg, 0.70 mmol) in ethanol (EtOH) (2.5 ml) was refluxed for 1 h and then cooled to room temperature. The reaction mixture was concentrated, extracted with EtOAc, washed with 1 N NaOH as well as H_2O, dried over $MgSO_4$, and concentrated in vacuo. The residue was used for the next step without purification. To an ice-cooled solution of this amine (50 mg, 0.16 mmol) in 2 ml of CH_2Cl_2 were added Et_3N (49.7 mg, 0.49 mmol) and isocyanate **12** (53 mg, 0.18 mmol), and the reaction mixture was kept at the same temperature for 2 h. The reaction mixture was concentrated, and the residue was extracted with $CHCl_3$, washed with H_2O, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a silica gel column using $CHCl_3$–acetone (2:1, v/v) as an eluent to give **35a′** (74 mg, 54%) as a powder. Softening point 103–105 °C. ^{1}H NMR ($CDCl_3$) δ: 1.73–2.04 (m, 6H), 2.34 (t, 2H, J = 7.4 Hz), 3.05 (t, 1H, J = 10 Hz), 3.13–3.25 (m, 2H), 3.36–3.54 (m, 4H), 3.65 (s, 3H), 3.71–3.82 (m, 1H), 4.05, 4.95 (ABq, 2H, J = 16.4 Hz, Δν = 181.7 Hz), 4.65–4.77 (m, 1H), 4.90 (s, 2H), 7.04–7.68 (m, 8H). Anal. calcd for $C_{29}H_{35}N_5O_7S$: C, 57.21; H, 6.24; N, 11.12; S, 5.09. Found: C, 56.93; H, 5.94; N, 11.16; S, 4.99.

(*S*)-4-(3-{3-[4-Oxo-5-(2-oxo-2-thiazolidin-3-ylethyl)-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid methyl ester (35b). Compound **35b** was prepared by the method used for compound **35a′**. Softening point 88–90 °C. ^{1}H NMR ($CDCl_3$) δ: 1.82 (q, 2H, J = 7 Hz), 2.36 (t, 2H, J = 7 Hz), 2.88–3.24 (m, 7H), 3.66 (s, 3H), 3.83 (t, 2H, J = 6 Hz), 4.03–4.14 (m, 1H), 4.45–4.76 (m, 2H), 4.91 (s, 2H), 4.99–5.09 (m, 1H), 7.10–7.67 (m, 8H). Anal. calcd for $C_{28}H_{33}N_5O_7S_2$: C, 54.61; H, 5.40; N, 11.38; S, 10.42. Found: C, 54.41; H, 5.61; N, 11.25; S, 10.21.

(*S*)-4-{3-[3-(5-*tert*-Butoxycarbonylmethyl-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl)ureido]benzyloxycarbonylamino)butyric acid methyl ester (35c). Compound **35c** was prepared by the method used for compounds **35a′** and **34a′** via compound **33**. Softening point 67–68 °C. ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H), 1.73–1.91 (m, 2H), 2.32–2.42 (m, 2H), 2.92–3.07 (m, 1H), 3.14–3.27 (m, 2H), 3.67 (s, 3H), 3.72–3.86 (m, 1H), 4.13, 4.72 (ABq, 2H, J = 17.2 Hz, Δν = 117.2 Hz), 4.67–4.81 (m, 1H), 4.92 (s, 2H), 6.83–7.71 (m, 8H). Anal. calcd for $C_{29}H_{36}N_4O_8S$: C, 57.98; H, 6.04; N, 9.33; S, 5.34. Found: C, 57.68; H, 6.10; N, 9.18; S, 5.05.

4-(3-{3-[5-(2-Furan-2-yl-2-oxoethyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid metyl ester (35d′). Compound **35d′** was prepared by the method used for compound **35a′**. Softening point 82–84 °C. ^{1}H NMR ($CDCl_3$) δ: 1.80 (q, 2H, J = 7 Hz), 2.34 (t, 2H, J = 7 Hz), 2.93 (t, 1H, J = 11 Hz), 3.18 (q, 2H, J = 6.4 Hz), 3.65 (s, 3H), 3.72–3.82 (m, 1H), 4.57, 5.54 (ABq, 2H, J = 17.6 Hz, Δν = 195.4 Hz), 4.73–4.83 (m, 1H), 4.95 (s, 2H), 6.48–6.53 (m, 1H), 7.07–7.66 (m, 9H), 7.64 (d, 1H, J = 7 Hz). Anal. calcd for $C_{29}H_{30}N_4O_8S{\cdot}0.5H_2O$: C, 57.69; H, 5.18; N, 9.28; S, 5.31. Found: C, 57.39; H, 5.19; N, 9.29; S, 5.19.

4-(3-{3-[4-Oxo-5-(2-oxo-2-pyrrolidin-1-ylethyl)-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid (36a′). Into an ice-cooled solution of **35a′** (464 mg, 0.78 mmol) in 5 ml of MeOH was added a solution of KOH (87 mg, 1.55 mmol) in 1 ml of H_2O with stirring. After stirring for 4 h at this temperature, this reaction mixture was neutralized with 1 N HCl and the solvent was evaporated in vacuo. The residue was extracted with EtOAc. The organic layer was washed with saturated sodium chloride (NaCl),

dried over $MgSO_4$ and concentrated. The residue (365 mg) was used for the next step without further purification. ^1H NMR ($CDCl_3$) δ: 1.69–2.00 (m, 6H), 2.30 (t, 2H, J = 6.8 Hz), 2.91 (t, 1H, J = 11 Hz), 3.08–3.20 (m, 2H), 3.46–3.60 (m, 4H), 3.68–3.78 (m, 1H), 4.09, 5.01 (ABq, 2H, J = 16.4 Hz, Δν = 186 Hz), 4.59–4.70 (m, 1H), 4.98 (s, 2H), 6.92–7.70 (m, 8H).

(*S*)-4-(3-{3-[4-Oxo-5-(2-oxo-2-thiazolidin-3-ylethyl)-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid (36b). Compound **36b** was prepared by the method used for compound **36a′**. ^1H NMR (CD_3OD) δ: 1.76 (q, 2H, J = 7.2 Hz), 2.31 (t, 2H, J = 7.4 Hz), 2.92 (t, 1H, J = 11.4Hz), 3.02–3.20 (m, 6H), 3.64–3.88 (m, 2H), 4.22–4.34 (m, 1H), 4.58–4.68 (m, 2H), 4.99 (s, 2H), 5.01–5.10 (m, 1H), 6.92–7.71 (m, 8H).

(*S*)-4-{3-[3-(5-*tert*-Butoxycarbonylmethyl-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl)ureido]benzyloxycarbonylamino)butyric acid (36c). Compound **36c** was prepared by the method used for compound **36a′**. ^1H NMR ($CDCl_3$) δ: 1.43 (s, 9H), 1.68–1.89 (m, 2H), 2.26–2.39 (m, 2H), 2.91–3.06 (m, 1H), 3.11–3.26 (m, 2H), 3.68–3.82 (m, 1H), 4.12, 4.72 (ABq, 2H, J = 16.8 Hz, Δν = 116.2 Hz), 4.65–4.79 (m, 1H), 4.95 (s, 2H), 6.81–7.71 (m, 8H).

4-(3-{3-[5-(2-Furan-2-yl-2-oxoethyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl]ureido}benzyloxycarbonylamino)butyric acid (36d′). Compound **36d′** was prepared by the method used for compound **36a′**. ^1H NMR (CD_3OD) δ: 1.76 (q, 2H, J = 7 Hz), 2.30 (t, 2H, J = 7 Hz), 2.94 (t, 1H, J = 11 Hz), 3.14 (t, 2H, J = 7.4 Hz), 3.69–3.78 (m, 1H), 4.73, 5.55 (ABq, 2H, J = 17.6 Hz, Δν = 164.3 Hz), 4.62–4.71 (m, 1H), 4.99 (s, 2H), 6.69–6.71 (m, 1H), 7.20–7.84 (m, 9H), 7.70 (d, 1H, J = 7 Hz).

Bioassay procedures

In vitro experiments

Binding assays for the gastrin,[10] CCK-B, and CCK-A receptors[11]. Guinea pig gastric glands (for gastrin binding) were suspended in binding assay buffer with [^{125}I]-gastrin and the appropriate concentration of unlabeled compounds. The suspensions were incubated at 25 °C for 30 min. Mouse brain cortex (for CCK-B binding) and pancreas membranes (for CCK-A binding) were suspended in binding assay buffer with [^{3}H]-CCK-8 and the appropriate concentration of unlabeled compounds. The suspensions were incubated at 25 °C for 90 min. Incubation was terminated by filtration through glass fiber GF/B filters and washing three times with buffer. Specific binding was defined as the difference between total binding and nonspecific binding in the presence of 2 μM gastrin or 1 μM CCK-8.

Binding assays for the histamine H_2 receptor. Evaluation was done by a well-established procedure.[12]

In vivo experiments

Gastric acid inhibitory activity: Antisecretory activities were studied using pylorus ligation preparations.[14] Under ether anesthesia, the abdomen was incised, the pylorus ligated, and histamine 2 HCl (3 mg kg^{-1}, sc) injected. The animal was killed 2 h later, and the gastric contents were collected and analyzed for volume and acidity. Acidity was determined by automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0 (autoburette). Titratable acid output was expressed as μEq 2 h^{-1}. Test compound or vehicle alone was given by po at 10 mg kg^{-1} at 1 h before ligating the pylorus.

Acknowledgements

We thank Dr M. Narisada (ex-director of the laboratories), Dr T. Yoshida, Dr S. Kamata, and Dr M. Doteuchi for their generous support and suggestions throughout the course of this work. We also thank very much Messrs K. Otani, F. Hirose, G. Kato, Mmes. N. Shima, and M. Shimamura for the biological assays.

References

1. Kawanishi, Y.; Ishihara, S.; Tsushima, T.; Seno, K.; Miyagoshi, M.; Hagishita, S.; Ishikawa, M.; Shima, N.; Shimamura, M.; Ishihara, Y. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1421.

2. Kawanishi, Y.; Ishihara, S.; Tsushima, T.; Seno, K.; Miyagoshi, M.; Hagishita, S.; Ishikawa, M.; Shima, N.; Shimamura, M.; Ishihara, Y. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1427.

3. Kawanishi, Y.; Ishihara, S.; Takahashi, K.; Tsushima, T.; Hagishita, S.; Ishikawa, M.; Ishihara, Y. *Chem. Pharm. Bull.* **1997**, *45*, 116.

4. Kawanishi, Y.; Ishihara, S.; Tsushima, T.; Hagishita, S.; Ishikawa, M.; Ishihara, Y. *Bioorg. Med. Chem.* **1997**, *5*, 1401.

5. Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Freidinger, R. M. *J. Med. Chem.* **1989**, *32*, 13.

6. (a) Lotti, V. J.; Chang, R. S. L. *Eur. J. Pharmacol.* **1989**, *162*, 273–280; (b) Murphy, M. J.; Sytnik, B.; Kovacs, T. O. G.; Mertz, H.; Ewanik, D.; Shingo, S.; Lin, J. H.; Gertz, B. J.; Walsh, J. H. *Clin. Pharmacol. Ther.* **1993**, *54*, 533.

7. Briggs, L. H.; De Ath, G. C. *J. Chem. Soc.* **1937**, 456.

8. Watthey, J. W. H.; Stanton, J. L.; Desai, M.; Babiarz, J. E.; Finn, B. M. *J. Med. Chem.* **1985**, *28*, 1511.

9. Itoh, K.; Kori, M.; Inada, Y.; Nishikawa, K.; Kawamatsu, Y.; Sugihara, H. *Chem. Pharm. Bull.* **1986**, *34*, 1128.

10. Chang, R. S. L.; Lotti, V. J.; Keegan, M. E.; Kunkel, K. A. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 895.

11. Saito, A.; Goldfine, I. D.; Williams, J. A. *J. Neurochem.* **1981**, *37*, 483.

12. Black, J. W.; Duncan, W. A. M.; Durant, C. J.; Ganellin, C. R.; Parsons, E. M. *Nature*, **1972**, *236*, 385.

13. All *Clog*P values were calculated by a computer program MedChem Ver. 3.54, DAYLIGHT Chemical Information Systems, Inc., USA.

14. Rat pylorus ligation method: Shay, H.; Komarov, S. A.; Fels, S. S.; Meranze, D.; Gruenstein, M.; Siplet, H. *Gastroenterology* **1945**, *5*, 43.

(*Received in Japan* 16 *January* 1997; *accepted* 31 *March* 1997)