Synthesis and Biological Evaluation of a New Reversely Linked Type of Dual Histamine H_2 and Gastrin Receptor Antagonist

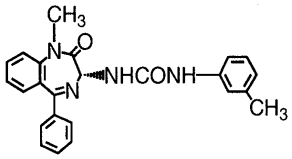
Yasuyuki KAWANISHI,*[,a] Shoichi ISHIHARA,[a] Kimio TAKAHASHI,[a] Tadahiko TSUSHIMA,*[,a] Sanji HAGISHITA,[a] Michio ISHIKAWA,[b] and Yasunobu ISHIHARA[b]

Discovery Research Laboratories II[a] and Development Research Laboratories,[b] Pharmaceuticals Research Divisions, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan. Received June 13, 1996; accepted July 29, 1996

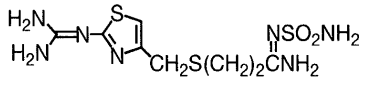
In an attempt to improve the low oral absorbability of previously reported dual histamine H_2 and gastrin receptor antagonists, compounds of a different type were synthesized and evaluated for biological activity. These new compounds bear a histamine H_2 receptor antagonist (H_2A) pharmacophore moiety attached to a gastrin receptor antagonist (GA) pharmacophore moiety in a reversed manner, namely the head-to-head manner, different from the previously reported head-to-tail manner. These new hybrid compounds were classified into three types: type I, the regular amide type bearing a roxatidine moiety; type II, the reversed amide type bearing a roxatidine moiety; and type III, hybrid compounds bearing a famotidine moiety directly connected to a GA moiety without a spacer. Among them, only (*R*)-1-[3-(*N'*-{4-[2-(*N*-aminosulfonylamidino)ethylthiomethyl]thiazol-2-yl}guanidinomethyl)phenyl]-3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)urea (42), belonging to type III, showed a weak but distinct histamine H_2 receptor-antagonistic activity as well as a modest gastrin receptor-antagonistic activity. Of most importance was the finding that this compound showed a weak but clearly improved *in vivo* oral antigastric acid secretory activity as a result of the structural changes, including the decreased molecular weight.

Key words histamine H_2 receptor antagonist; gastrin receptor antagonist; anti-ulcer agent

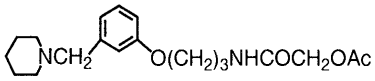
With the objective of alleviating the relapse problem[1] frequently encountered in the chemotherapy of peptic ulceration with histamine H_2 receptor antagonists (H_2A), we have conducted a series of studies on the design, synthesis and pharmacological evaluation of dual histamine H_2 and gastrin receptor antagonists. In our previous papers,[2,3] we reported for the first time, to our knowledge, the synthesis and evaluation of some joint types of hybrid molecules which had been constructed from two basic pharmacophore moieties selected from a well-known H_2A [either famotidine[4] or roxatidine[5]] and a gastrin receptor antagonist (GA) [L-365,260[6]]. In these molecules, the two pharmacophores were connected with a spacer so as to attach the GA moiety to the tail amine part of the H_2A moiety, as shown in Fig. 1. Those studies showed that the presence of a spacer as well as the selection of spacer binding sites, particularly for the GA moiety, was very important for retaining both the H_2A and GA



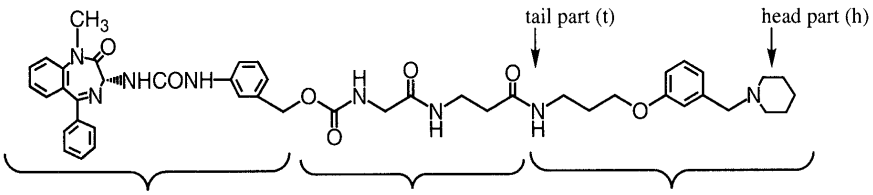
L-365,260



famotidine



roxatidine acetate



compound **1**

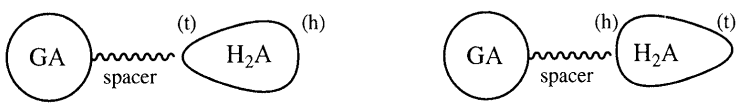


Fig. 1

* To whom correspondence should be addressed.

activities. Alteration of spacers as well as their binding modes at the GA site, *e.g.* from the amide type to the carbamate type, significantly improved not only the H_2A and GA activities, but also the GA *versus* cholecystokinin-A (CCK-A) receptor selectivity.

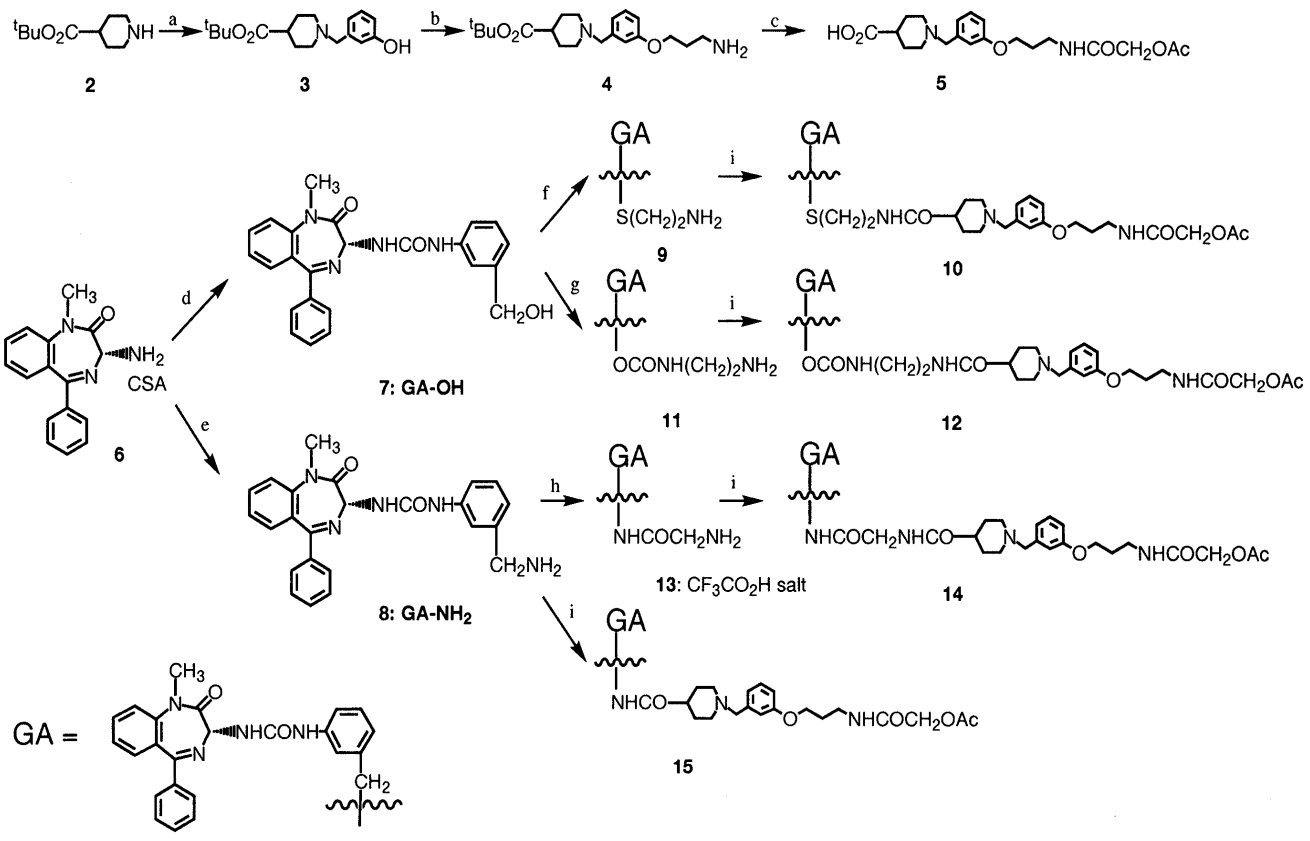
The most active compound of all the carbamate-type compounds prepared was hybrid compound **1**, which showed a pA_2 value of 6.8 for the histamine H_2 receptor and an IC_{50} value of 19 nM for the gastrin receptor. This compound passed our *in vitro* activity standards set for selecting compounds for *in vivo* biological evaluation. Furthermore, its *in vivo* gastric acid antisecretory activities measured by both Schild's rat method[7] and the Heidenhain pouch dog method[8] were excellent as long as the drug was administered by the i.v. route. When compound **1** was administered by the i.d. or oral route, its activities were much weaker, clearly indicating low oral absorbability. It became obvious from those studies that improvement of the oral absorbability of these hybrid molecules would be essential for their development as anti-ulcer agents for practical use. We thus decided to try to alter the molecular polarity of these dual antagonists and also to reduce their molecular weight.

We report here a different type of molecular design from our previous one. We synthesized and evaluated reversely linked hybrid molecules in which the GA moiety is connected to the head heterocyclic part of an H_2A moiety. We also tried to decrease the molecular weight of these new hybrid molecules.

Chemistry

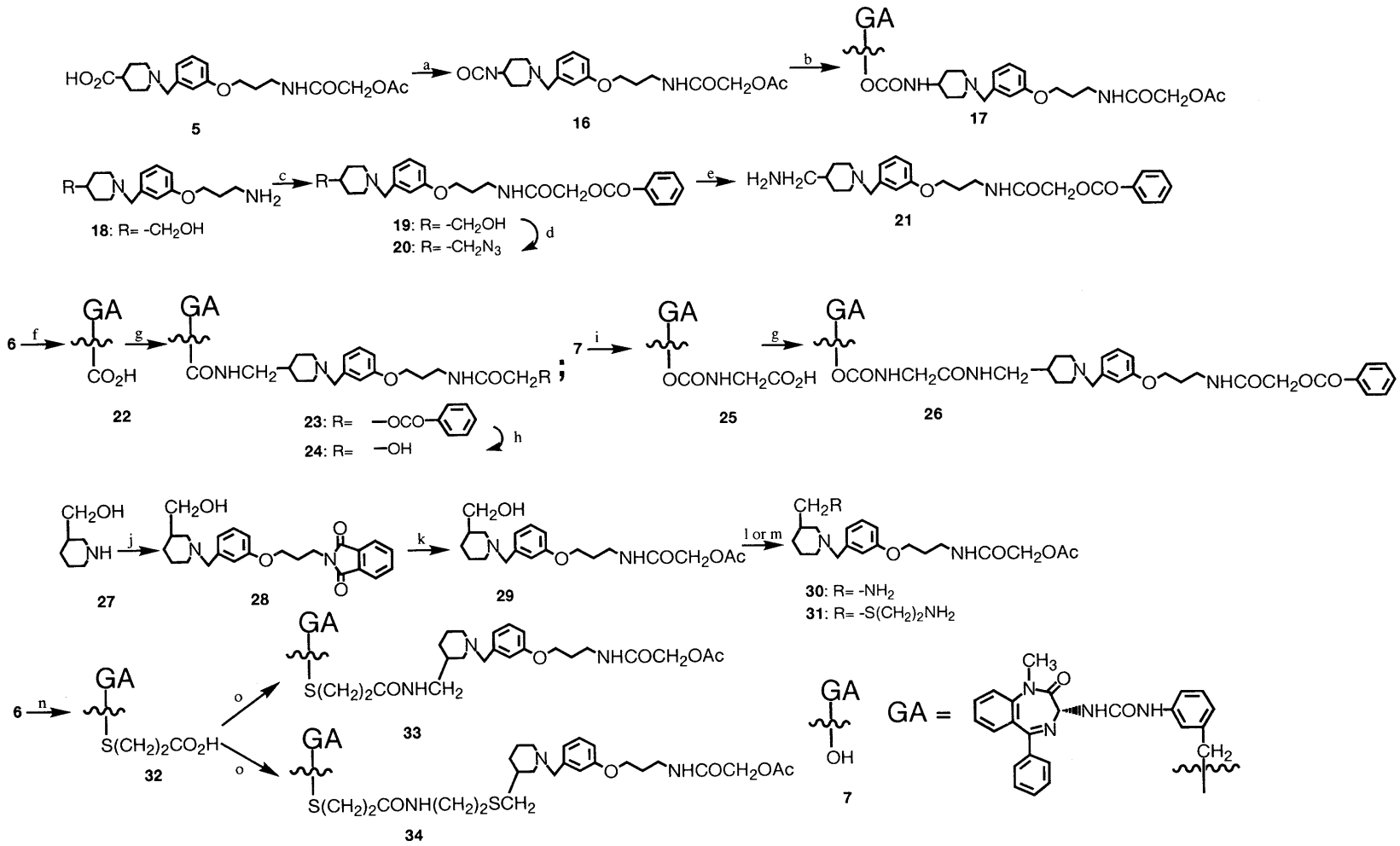
The reversely linked hybrid compounds we studied can be classified into three types: (1) Type I is a normal amide type bearing a roxatidine moiety reversely connected to a GA moiety at the C_4 position of the piperidine ring by a spacer, (2) type II is a reversed amide type bearing a roxatidine moiety reversely connected to a GA moiety with a spacer either at the C_4 or C_3 positions of the piperidine ring, and (3) type III is a direct type of hybrid bearing a famotidine moiety reversely connected, without any spacer, to a GA moiety at the guanidino group on the thiazole ring. The molecular structures and synthetic schemes of these type I, II, and III compounds are summarized in Charts 1, 2, and 3, respectively.

The basic strategy adopted for the synthesis of type I compounds (Chart 1) was to couple an amino derivative of L-365,260 with a carboxylic acid derivative of roxatidine **5**, prepared as follows. The tertiary butyl ester of isonipecotic acid **2** was prepared from commercially available isonipecotic acid *via* *N*-benzyloxycarbonylation, esterification, and *N*-deprotection. Next, **2** was treated with 3-hydroxybenzaldehyde and sodium borohydride to give compound **3**. After alkylation of the phenolic hydroxyl group with *N*-(3-bromopropyl)phthalimide the intermediate obtained was treated with hydrazine monohydrate to give compound **4**. The resulting amine **4** was acylated with acetoxyacetyl chloride and then converted to the desired free carboxylic acid **5** by treatment with trifluoroacetic acid. The GA components used in



a: 3-hydroxybenzaldehyde, $NaBH_4$ b: 1) 2-(3-bromopropyl)isoindole-1,3-dione 2) hydrazine monohydrate c: 1) acetoxyacetyl chloride 2) CF_3CO_2H d: 1) 3-acetoxymethylphenyl isocyanate 2) aq.KOH/MeOH e: 1) 3-(trifluoroacetylaminomethyl)phenyl isocyanate 2) aq.K_2CO_3/MeOH f: 1) $BocNH(CH_2)_2SH$ 2) 4N HCl/AcOEt g: 1) chloroethyl isocyanate 2) Mitsunobu reaction HN_3 3) $(C_6H_5)_3P$ h: 1) HOBt, Et_3N, WSCI, $BocNHCH_2CO_2H$ 2) CF_3CO_2H i: HOBt, Et_3N, WSCI, **5**

Chart 1



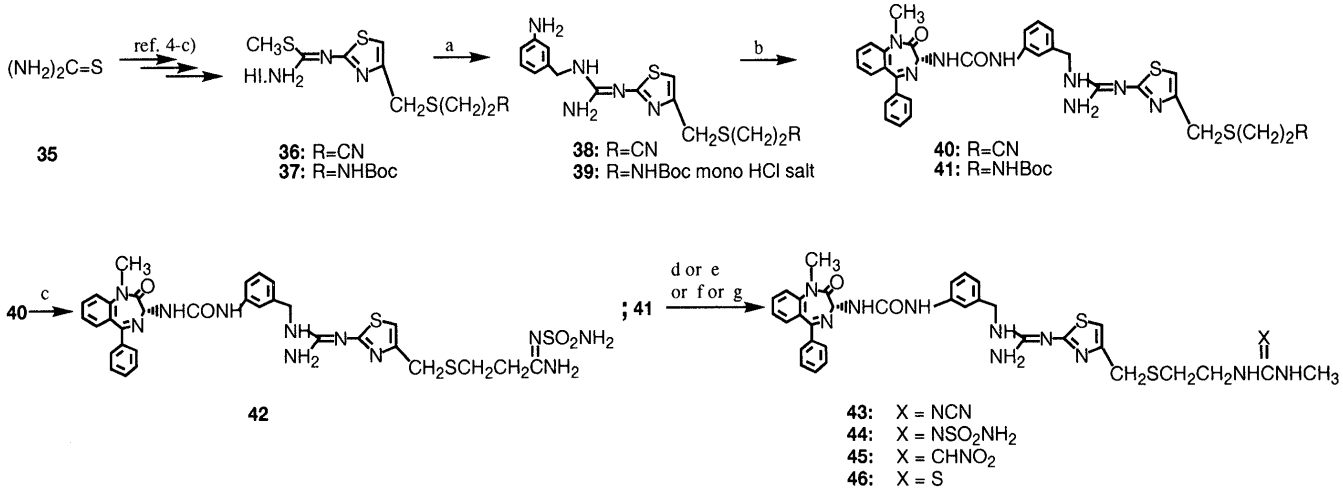
a: 1) $ClCO_2Et$, Et_3N 2) NaN_3 3) heat b: $(n\text{-}Bu_3Sn)_2O$, **7** c: HOBt, Et_3N, WSCI, $C_6H_5CO_2CH_2CO_2H$ d: Mitsunobu reaction HN_3 e: $(C_6H_5)_3P$ f: 1) (3-isocyanatophenyl)acetic acid ethyl ester 2) aq.NaOH/EtOH g: HOBt, Et_3N, WSCI, **21** h: aq.NaOH/EtOH i: 1) $OCNCH_2CO_2Et$, $(n\text{-}Bu_3Sn)_2O$ 2) aq.NaOH/EtOH j: 1) 3-hydroxybenzaldehyde, $NaBH_4$ 2) 2-(3-bromopropyl)isoindole-1,3-dione k: 1) hydrazine monohydrate 2) acetoxyacetyl chloride l: 1) Mitsunobu reaction HN_3 2) $(C_6H_5)_3P$ m: 1) $BocNH(CH_2)_2SH$ 2) CF_3CO_2H n: 1) 3-(3-isocyanatobenzylthio)propionic acid methyl ester 2) aq.NaOH/EtOH o: HOBt, Et_3N, WSCI, **30** or **31**

Chart 2

the coupling reaction with **5**, the hydroxyl- and amino-substituted benzodiazepine derivatives, **7** and **8**, were prepared from the known optically active agent, 3-amino-1-methyl-5-phenyl-1,4-benzodiazepine **6**[9] by treatment with the corresponding isocyanates, 3-acetoxymethylphenyl isocyanate and 3-(trifluoroacetylaminomethyl)phenyl isocyanate, followed by deprotection of the hydroxyl and the amino group, respectively. The benzodiazepine amino derivative **9** was prepared from **7** by Mitsunobu reaction with *N*-Boc-cysteamine, followed by *N*-deprotection. The other derivative **11** was also prepared from **7** *via* treatment with 2-chloroethyl isocyanate in the presence of bis(tri-*n*-butyltin) oxide as a catalyst, azidation, and reduction with triphenylphosphine. Another amine derivative **13** was prepared by coupling **8** with *N*-Boc-glycine in the usual manner using water-soluble carbodiimide as a coupling agent, followed by *N*-deprotection. All these amine derivatives **9**, **11**, **13**, **8** were coupled with the carboxylic acid **5**, giving the hybrid compounds **10**, **12**, **14**, **15**, respectively, in good to moderate yields.

Type II compounds with reversed amide bonding were synthesized as shown in Chart 2. First, hybrid compound **17** bearing the carbamate bond at the 4-position of piperidine was synthesized as follows. The starting material, 4-carboxylroxatidine acetate **5**, prepared as shown in Chart 1, was converted into the isocyanate derivative **16** by Curtius reaction under usual conditions. Next, **16** was allowed to react with benzodiazepine alcohol derivative **7** in the presence of bis(tri-*n*-butyltin) oxide as a catalyst to afford the desired hybrid compound **17**. Hybrid compounds **23** and **26**, bearing the reversed amide bond at the C_4 position of piperidine, were synthesized as follows. The amine **21** was prepared by coupling **18** with benzoyloxyacetic acid followed by Mitsunobu reaction with hydrazoic acid and reduction of the resultant azide product with triphenylphosphine. Benzodiazepinecarboxylic acid intermediates **22** and **25** were prepared from the amine **6** and the alcohol **7** by treatment with the corresponding isocyanates, (3-isocyanatophenyl)acetic acid ethyl ester and isocyanatoacetic acid ethyl ester, respectively, followed by hydrolysis. The coupling reactions of these carboxylic acids with **21** gave the desired hybrid compounds **23** and **26** in moderate to good yields. The free alcohol derivative **24** was prepared by saponification of **23**. Next, type II hybrid compounds with the reversed amide bond at the C_3 position of the piperidine ring were synthesized as follows. 3-Hydroxymethylpiperidine **27** was allowed to react, in sequence, with 3-hydroxybenzaldehyde and *N*-(3-bromopropyl)phthalimide under the same conditions used for the synthesis of compound **3** shown in Chart 1 to give compound **28**. After dephthalization with hydrazine monohydrate, the resulting amine was acylated with acetoxyacetyl chloride under the same conditions as used for compound **4** in Chart 1, giving compound **29** in a moderate yield. This compound was converted into the aminomethyl derivative **30** by Mitsunobu reaction with HN_3 followed by reduction with triphenylphosphine. The other amine derivative **31** which has a sulfide bond in a spacer part was prepared from **29** by Mitsunobu reaction with *N*-Boc-cysteamine followed by *N*-deprotection. Meanwhile, the benzodiazepine carboxylic acid derivative **32** was prepared from **6** by treatment with isocyanate 3-(3-isocyanatobenzylthio)-propionic acid methyl ester which was prepared from *N*-Boc-3-bromomethylphenylamine in 3 steps, followed by hydrolysis. Compound **32** was coupled with amines **30** and **31** to yield hybrid compounds **33** and **34**.

Type III hybrid compounds bearing a famotidine moiety reversely connected to a GA moiety without any spacer were synthesized as shown in Chart 3. For example, the hybrid compound with a lower molecular weight **42** was synthesized as follows. The thiourea **35** was converted to the (*S*-methylisothioureido)thiazole **36** and **37** in 5 steps by reported methods.[4c] They were allowed to react with 3-aminobenzylamine to give benzylamine-substituted compounds **38** and **39**, respectively. Compound **38** gave the nitrile **40** in a moderate yield on treatment with optically active 3-isocyanato-1-methyl-5-phenyl-1,4-benzodiazepine prepared *in situ* from optically active



a: 3-aminobenzylamine or 1) 3-aminobenzylamine 2) 1eq. 4N HCl/AcOEt b: 3S-isocyanato-1-methyl-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one c: HCl-MeOH and $NH_2SO_2NH_2$ d: 1) 4N HCl/AcOEt 2) $(C_6H_5O)_2C{=}NCN$, Et_3N 3) CH_3NH_2 e: 1) 4N HCl/AcOEt 2) $(C_6H_5O)_2C{=}NSO_2NH_2$, Et_3N 3) CH_3NH_2 f: 1) 4N HCl/AcOEt 2) $(CH_3S)_2C{=}CHNO_2$, Et_3N 3) CH_3NH_2 g: 1) 4N HCl/AcOEt 2) CH_3NCS, Et_3N

Chart 3

3-amino-1-methyl-5-phenyl-1,4-benzodiazepine and triphosgene. Compound **40** was carefully converted to the desired sulfamoyl amidine **42** by successive treatments with gaseous hydrogen chloride in methanol at low temperature, with potassium carbonate, and with sulfamide as described in the literature.[4c,d] For the synthesis of hybrid compounds with modified guanidines, **43** and **44**, as well as those with modified amidines, **45** and **46**, the key intermediate *N*-Boc amine **41** was prepared in the same way as that used for **40**, as shown in Chart 3. The hybrid compounds **43**—**45** were prepared by treatment of **41** with diphenyl cyanocarbonimidate, with diphenyl *N*-sulfamoylcarbonimidate, and with 1,1-dithiomethyl-2-nitroethane, respectively, followed by treatment of the intermediate with methylamine. The thiourea hybrid compound **46** was prepared in one step by treatment of **41** with methyl isothiocyanate.

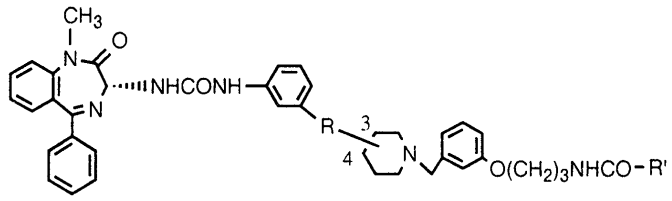
Biological Results and Discussion

The *in vitro* biological activities[10-12] and the chemical structures of the reversely linked hybrid compounds of type I (the amide type) and type II (the reversed amide type) are summarized in Table 1. All these compounds contain a roxatidine pharmacophore moiety connected with a spacer to the C3′ position of L-365,260, in a reversed manner, namely at the head heterocyclic positions. However, they differ from each other in the binding mode of the spacer, the former having the normal amide bond and the latter the reversed amide bond. The biological results show that the pA_2 values of most of these reversely linked amide type, as well as reversed amide type, hybrid compounds listed in Table 1, *e.g.* **12**, **14**, **15**, **17**, **23**, **24**, and **26**, were extremely low, with values in the range of 5.0—5.3, irrespective of the differences in the binding mode or the length of the spacer. Exceptionally, the compounds with sulfide bonding (**10**, **33**, **34**) showed slightly higher pA_2 values of 5.5—5.9. However, no further improvement was achieved in these cases either by alteration of the binding mode of the spacers or their binding sites at the piperidine ring. Thus, these low pA_2 values suggest that the H_2A activity of the hybrid compounds is greatly decreased in going from the previously reported ones (for example, compound **1**) to these reversely linked ones. However, unlike the H_2A activities, the GA activities of all these reversely linked hybrid compounds are relatively well retained, with approximately one-tenth of the potency of L-365,260, and the GA *versus* CCK-A receptor selectivities are also fairly high, as shown by compounds **10**, **17**, and **26**. In particular, compound **10** exhibited almost the same GA activity and receptor selectivity as those of compound **1**.

Table 2 summarizes the *in vitro* biological activities[10-12] of the hybrid compounds with a famotidine moiety reversely connected to L-365,260, together with their chemical structures. Compound **42**, which bears the famotidine moiety reversely connected to L-365,260 at the guanidino group without a spacer showed a somewhat higher pA_2 value of 6.1 than the roxatidine cases described above. However, as shown by compounds **43**—**46** chemical modification of the amidino group in **42** failed to improve its pA_2 value. On the other hand, with respect to GA activity, **42** showed one-tenth of the potency of L-365,260, namely $IC_{50}=38$ nM, which is comparable to those of the roxatidine derivatives. As shown by compound **44**, the chemical modification of the amidino group of **42** slightly improved its GA activity as well as the GA *versus* CCK-A receptor selectivity.

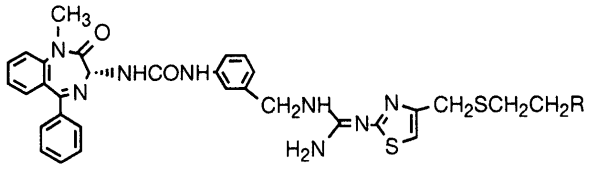
Our attention was particularly directed towards com-

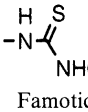
Table 1. *In Vitro* Biological Activities of Type I and II Hybrid Compounds and Derivatives



No.	R	Position 3 or 4	R′	Receptors IC_{50} (nM)			Ratio Gastrin / CCK-A	pA_2
				Gastrin	CCK-B	CCK-A		Histamine H_2
10	$CH_2S(CH_2)_2NHCO$	4	CH_2OAc	20	130	7600	380	5.5
12	$CH_2OCONH(CH_2)_2NHCO$	4	CH_2OAc	36	430	1300	36	<5.0
14	$CH_2NHCOCH_2NHCO$	4	CH_2OAc	32	270	300	9	<5.0
15	CH_2NHCO	4	CH_2OAc	9	37	135	15	5.3
17	CH_2OCONH	4	CH_2OAc	9	60	940	104	5.1
23	$CH_2CONHCH_2$	4	CH_2OCOPh	55	230	340	6	<5.0
24	$CH_2CONHCH_2$	4	CH_2OH	100	360	94	1	5.0
26	$CH_2OCONHCH_2CONHCH_2$	4	CH_2OCOPh	29	180	5400	186	5.0
33	$CH_2S(CH_2)_2CONHCH_2$	3	CH_2OAc	57	93	120	2	5.7
34	$CH_2S(CH_2)_2CONH(CH_2)_2SCH_2$	3	CH_2OAc	81	220	2700	33	5.9
9	$CH_2S(CH_2)_2NH_2$			56	420	2700	48	
13	$CH_2NHCOCH_2NH_2CF_3CO_2H$			40	640	360	9	
1				19	103	8200	432	6.8
	Roxatidine acetate							7.2
	L-365,260			4	29	11100	2775	

Table 2. *In Vitro* Biological Activities of Type III Hybrid Compounds



No.	R	Receptors IC_{50} (nM)			Ratio Gastrin / CCK-A	pA_2
		Gastrin	CCK-B	CCK-A		His. H_2
42		38	270	560	15	6.1
43		105	760	260	3	5.7
44		16	340	350	22	5.7
45		220	320	320	2	6.0
46		120	420	410	3	5.2
	Famotidine					7.3
	L-365,260	4	29	11100	2775	

pound **42**, as it had one of the lowest molecular weights among the hybrid compounds synthesized and contained no spacer, but showed a distinct pA_2 value of 6.1. All previously reported joint-type hybrid compounds had shown no distinct pA_2 values when they lacked a spacer.[2] In addition, compound **42** was supposed to have a somewhat different molecular polarity from those of the previously reported molecules owing to differences in the connection mode of the H_2A and the GA moiety. We thus expected that compound **42** would show a somewhat better oral absorbability than compound **1**. In fact, its *in vivo* gastric acid antisecretory activity evaluated by the rat pylorus ligation method[13] at 10 mg/kg *via* the oral route revealed that compound **42** exhibited a higher inhibitory activity, 69%, than that of compound **1**, 54%, suggesting improved oral absorbability. For practical use, however, this value is still unsatisfactory. Furthermore, no dose–response relationship was observed. We are conducting further studies to improve the oral absorbability of hybrid compounds with dual H_2A and GA activities, and will report the results in due course.

Experimental

All melting points and softening points were determined on a Yanagimoto micromelting point apparatus without correction. IR spectra were recorded on a Hitachi 260-10 IR spectrophotometer. The ^{1}H-NMR spectra were taken on a Varian VXR-200 spectrometer for organic solutions using tetramethylsilane (TMS) as an internal standard and chemical shifts are 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. (*R*)-(3-{3-[4-(2-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzylthio}ethylcarbamoyl)piperidin-1-yl]methylphenoxy}propylcarbamoyl)methyl Acetate (10). **Method A** 1-Hydroxybenzotriazole (HOBt) (20 mg, 0.146 mmol), triethylamine (Et_3N) (15 mg, 0.146 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCI) (28 mg, 0.146 mmol) were added in portions to a well stirred solution containing the carboxylic acid derivative of roxatidine **5** (48 mg, 0.122 mmol) and benzodiazepine amine **9** (60 mg, 0.122 mmol) in 2 ml of dimethylformamide (DMF) under ice cooling. The reaction mixture was stirred at room temperature for 16 h and concentrated under vacuum. The residue was extracted with methylene chloride (CH_2Cl_2) and washed with aqueous sodium hydrogen carbonate ($NaHCO_3$) and water (H_2O), then dried over magnesium sulfate ($MgSO_4$) and concentrated under vacuum. The residue was chromatographed on a silica gel column with chloroform ($CHCl_3$)–methanol (MeOH) (93 : 7, v/v) to give **10** (40 mg, 39%) as a powder. Softening point 168—170 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.60—2.15 (m, 8H, $4CH_2$), 2.15 (s, 3H, CH_3), 2.55 (t, 2H, CH_2, J=6 Hz), 2.85—3.00 (m, 2H, CH_2), 3.25—3.63 (m, 7H, $3CH_2$+CH), 3.49 (s, 3H, N–CH_3), 3.66 (s, 2H, CH_2), 4.02 (t, 2H, CH_2, J=6 Hz), 4.55 (s, 2H, CH_2), 5.49 (s, 1H, CH), 6.75—7.65 (m, 17H, Ar-H). *Anal.* Calcd for $C_{46}H_{53}N_7O_7S\cdot 1.0H_2O$: C, 63.79; H, 6.40; N, 11.32; S, 3.70. Found: C, 63.60; H, 6.32; N, 11.30; S, 3.59. Other type I and II hybrid compounds (**12**, **14**, **15**, **23**, **24**, **26**, **33**, **34**), but not compound **17**, were similarly obtained. The softening points, optical rotations, NMR, and analytical data were as follows.

(*R*)-(3-{3-[4-(2-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzyloxycarbonylamino}ethylcarbamoyl)piperidin-1-ylmethyl]phenoxy}propylcarbamoyl)methyl Acetate (12) ^{1}H-NMR ($CDCl_3$) δ: 1.60—2.25 (m, 8H, $4CH_2$), 2.11 (s, 3H, CH_3), 2.85—3.05 (m, 2H, CH_2), 3.20—3.40 (m, 9H, $4CH_2$+CH), 3.49 (s, 3H, N–CH_3), 3.53 (s, 2H, CH_2), 4.02 (t, 2H, CH_2, J=5.8 Hz), 4.34 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 5.48 (s, 1H, CH), 6.75—7.65 (m, 17H, Ar-H). *Anal.* Calcd for $C_{47}H_{54}N_8O_9\cdot 0.5H_2O$: C, 63.86; H, 6.27; N, 12.68. Found: C, 64.10; H, 6.35; N, 12.86.

(*R*)-[3-(3-{4-[({3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzylcarbamoyl}methyl)carbamoyl]piperidin-1-ylmethyl}phenoxy)propylcarbamoyl]methyl Acetate (14) ^{1}H-NMR ($CDCl_3$) δ: 1.65—2.35 (m, 8H, $4CH_2$), 2.15 (s, 3H, CH_3), 2.90—3.02 (m, 2H, CH_2), 3.38—3.60 (m, 5H, $2CH_2$+CH), 3.49 (s, 3H, N–CH_3), 3.88 (s, 2H, CH_2), 4.05 (t, 2H, CH_2, J=6 Hz), 4.36 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 5.48 (s, 1H, CH), 6.75—7.65 (m, 17H, Ar-H). *Anal.* Calcd for $C_{46}H_{52}N_8O_8\cdot 2.0H_2O$: C, 62.71; H, 6.41; N, 12.72. Found: C, 62.96; H, 6.41; N, 12.77.

(*R*)-{3-[3-(4-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzylcarbamoyl}piperidin-1-ylmethyl)phenoxy]-propylcarbamoyl}methyl Acetate (15) ^{1}H-NMR ($CDCl_3$) δ: 1.70—2.20 (m, 8H, $4CH_2$), 2.13 (s, 3H, CH_3), 2.80—2.92 (m, 2H, CH_2), 3.35—3.60 (m, 9H, $3CH_2$+N–CH_3), 4.03 (t, 2H, CH_2, J=5.8 Hz), 4.35 (d, 2H, CH_2, J=6.5 Hz), 4.55 (s, 2H, CH_2), 5.45 (d, 1H, CH, J=8 Hz), 6.75—7.65 (m, 17H, Ar-H). *Anal.* Calcd for $C_{44}H_{49}N_7O_7$: C, 67.07; H, 6.27; N, 12.44. Found: C, 66.78; H, 6.36; N, 12.70.

(+)-(*R*)-[3-(3-{4-[(2-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]phenyl}acetylamino)methyl]piperidin-1-ylmethyl}phenoxy)propylcarbamoyl]methyl Benzoate (23) Softening point 130—132 °C. $[\alpha]_D^{25}$ +6.2° (c=1.008, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.17—2.09 (m, 9H, $4CH_2$+CH), 2.76—2.88 (m, 2H, CH_2), 3.01—3.17 (m, 2H, CH_2), 3.28—3.60 (m, 2H, CH_2), 3.40 (s, 2H, CH_2), 3.45 (s, 3H, N–CH_3), 3.51 (s, 2H, CH_2), 4.06 (m, 2H, CH_2), 4.84 (s, 2H, CH_2), 5.52 (d, 1H, 3-H, J=8 Hz), 6.82—8.10 (m, 17H, Ar-H). *Anal.* Calcd for $C_{50}H_{53}N_7O_7\cdot 3.0H_2O$: C, 65.41; H, 6.48; N, 10.68. Found: C, 65.45; H, 6.29; N, 10.83.

(−)-(*R*)-*N*-(1-{3-[3-(2-Hydroxyacetylamino)propoxy]benzyl}piperidine-4-ylmethyl)-2-{3-[3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]phenyl}acetamide (24) Softening point 118—120 °C. $[\alpha]_D^{25}$ −1.0° (c=1.021, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 1.18—2.11 (m, 9H, $4CH_2$+CH), 2.83—2.99 (m, 2H, CH_2), 3.02—3.14 (m, 2H, CH_2), 3.28—3.62 (m, 2H, CH_2), 3.50 (s, 2H, CH_2), 3.44 (s, 3H, N–CH_3), 3.74 (s, 2H, CH_2), 4.07 (m, 2H, CH_2), 4.84 (s, 2H, CH_2), 5.52 (d, 1H, 3-H, J=8 Hz), 6.82—8.10 (m, 17H, Ar-H). *Anal.* Calcd for $C_{43}H_{49}N_7O_6\cdot 3.6H_2O$: C, 62.61; H, 6.87; N, 11.89. Found: C, 62.90; H, 6.69; N, 11.70.

(−)-(*R*)-[3-(3-{4-[(2-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzyloxycarbonylamino}acetylamino)-methyl]piperidin-1-ylmethyl}phenoxy)propylcarbamoyl]methyl Benzoate (26) Softening point 117—119 °C. $[\alpha]_D^{25}$ −1.7° (c=1.005, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 1.19—2.10 (m, 9H, $4CH_2$+CH), 2.81—2.92 (m,

2H, CH_2), 2.99—3.11 (m, 2H, CH_2), 3.48—3.60 (m, 4H, $2CH_2$), 3.73—3.79 (m, 2H, CH_2), 3.42 (s, 3H, N–CH_3), 4.03—4.10 (m, 2H, CH_2), 4.84 (s, 2H, CH_2), 5.02 (s, 2H, CH_2), 5.51 (d, 1H, 3-H, J=8.2 Hz), 6.76—8.07 (m, 22H, Ar-H). *Anal.* Calcd for $C_{52}H_{56}N_8O_9 \cdot 1.2H_2O$: C, 65.15; H, 6.14; N, 11.69. Found: C, 65.23; H, 6.21; N, 11.39.

(*R*)-[3-(3-{3-[(3-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzylthio}propionylamino)methyl]piperidin-1-ylmethyl}phenoxy)propylcarbamoyl]methyl Acetate (33) ^{1}H-NMR ($CDCl_3$) δ: 1.55—2.35 (m, 8H, $4CH_2$), 2.14 (s, 3H, CH_3), 2.63 (t, 2H, CH_2, J=6 Hz), 2.80—3.10 (m, 4H, $2CH_2$), 3.30—3.70 (m, 7H, $3CH_2$+CH), 3.49 (s, 3H, N–CH_3), 3.65 (s, 2H, CH_2), 4.04 (t, 2H, CH_2, J=5.8 Hz), 4.54 (s, 2H, CH_2), 5.48 (s, 1H, CH), 6.75—7.65 (m, 17H, Ar-H). *Anal.* Calcd for $C_{47}H_{55}N_7O_7S \cdot 3.0H_2O$: C, 61.62; H, 6.71; N, 10.70; S, 3.50. Found: C, 61.35; H, 6.78; N, 10.56; S, 3.50.

(*R*)-[3-(3-{3-[2-(3-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzylthio}propionylamino)ethylthiomethyl]piperidin-1-ylmethyl}phenoxy)propylcarbamoyl]methyl Acetate (34) ^{1}H-NMR ($CDCl_3$) δ: 1.45—3.00 (m, 18H, $9CH_2$), 2.14 (s, 3H, CH_3), 3.46 (s, 3H, N-CH_3), 3.40—3.60 (m, 5H, $2CH_2$+CH), 3.65 (s, 2H, CH_2), 4.05 (t, 2H, CH_2, J=5.8 Hz), 4.59 (s, 2H, CH_2), 5.55 (d, 1H, CH, J=8 Hz), 6.70—7.63 (m, 17H, Ar-H). *Anal.* Calcd for $C_{49}H_{59}N_7O_7S_2 \cdot 1.5H_2O$: C, 62.00; H, 6.58; N, 10.33; S, 6.76. Found: C, 62.00; H, 6.63; N, 10.26; S, 6.66.

(*R*)-{3-[3-(4-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzyloxycarbonylamino}piperidin-1-ylmethyl)-phenoxy]propylcarbamoyl}methyl Acetate (17). Method B Bis(tributyltin) oxide (0.127 ml, 0.25 mmol) was added to a solution of the isocyanate **16** [prepared from the carboxylic acid **5** (98 mg, 0.25 mmol)] and **7** (104 mg, 0.25 mmol) in 2 ml of tetrahydrofuran (THF), and the reaction mixture was stirred at room temperature for 1 h, then concentrated. The residue was washed with diethyl ether (Et_2O) and chromatographed on silica gel with $CHCl_3$–MeOH (10 : 1, v/v) to give **17** (53 mg, 26%) as a powder. ^{1}H-NMR ($CDCl_3$) δ: 1.40—2.30 (m, 8H, $4CH_2$), 2.13 (s, 3H, CH_3), 3.47—3.65 (m, 5H, $2CH_2$+CH), 3.45 (s, 3H, N–CH_3), 4.05 (t, 2H, CH_2, J=6 Hz), 4.56 (s, 2H, CH_2), 4.98 (s, 2H, CH_2), 5.54 (d, 1H, CH, J=8 Hz), 6.62—7.70 (m, 17H, Ar-H). *Anal.* Calcd for $C_{44}H_{49}N_7O_8 \cdot 1.5H_2O$: C, 63.60; H, 6.31; N, 11.80. Found: C, 63.99; H, 6.22; N, 11.77.

(*R*)-1-[3-(*N'*-{4-[2-(*N*-Aminosulfonylamidino)ethylthiomethyl]thiazol-2-yl}guanidinomethyl)phenyl]-3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)urea (42). Method C An ice-cooled solution of **40** (2.94 g) in MeOH (60 ml) was bubbled through with hydrogen chloride gas. After 3 d in a refrigerator, the reaction mixture was concentrated *in vacuo*. The residue was taken up in aqueous potassium carbonate (K_2CO_3) and the product was extracted with $CHCl_3$. The organic solution was dried and concentrated by evaporation to afford the intermediate iminoether (3.30 g, 92%). ^{1}H-NMR ($CDCl_3$) δ: 2.40—2.75 (m, 4H, $2CH_2$), 3.42 (s, 3H, N–CH_3), 3.62 (s, 2H, CH_2), 3.69 (s, 3H, OCH_3), 4.29—4.35 (m, 2H, CH_2), 5.48 (d, 1H, 3-H, J=8 Hz), 6.37 (s, 1H, CH), 6.83 (d, 1H, NH, J=7 Hz), 7.08—7.61 (m, 13H, Ar-H).

The intermediate iminoether (3.0 g) was dissolved in dry MeOH (20 ml), and sulfamide (4.1 g, 42.7 mmol) was added. After 60 h at room temperature, the reaction mixture was concentrated and the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (20 : 1, v/v). The desired fractions were collected and concentrated under vacuum to give a solid residue, which was pulverized from H_2O to give **42** (1.00 g, 30%). Softening point 172—174 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.47 (t, 2H, CH_2, J=8 Hz), 2.78 (t, 2H, CH_2, J=8 Hz), 3.47 (s, 3H, N–CH_3), 3.65 (s, 2H, CH_2), 4.43 (s, 2H, CH_2), 5.38 (s, 1H, 3-H), 6.57 (s, 1H, thiazole-CH), 6.95-7.80 (m, 13H, Ar-H). *Anal.* Calcd for $C_{32}H_{35}N_{11}O_4S_3 \cdot 1.0H_2O$: C, 51.12; H, 4.96; N, 20.49; S, 12.79. Found: C, 51.36; H, 5.06; N, 20.23; S, 12.65.

(−)-(*R*)-1-[3-(*N'*-{4-[2-(*N*-Methyl-*N'*-cyanoguanidino)ethylthiomethyl]thiazol-2-yl}guanidinomethyl)phenyl]-3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)urea (43). Method D An ice-cooled solution of *N*-protected **41** (1.35 g, 1.85 mmol) in ethyl acetate (EtOAc) (50 ml) was added to an ice-cooled solution of 4 N hydrogen chloride in EtOAc (50 ml) with stirring and the reaction mixture was stirred at room temperature for 2 h. After evaporation of the solvent, EtOAc (200 ml) was added to the residue and evaporated under vacuum. This operation was repeated twice. The resulting crude amine 2HCl salt (1.35 g, quant.) was used for the next step. The amine 2HCl salt (200 mg, 0.285 mmol) was dissolved in 4 ml of MeOH, then diphenyl cyanocarbonimidate (75 mg, 0.315 mmol) and Et_3N (0.039 ml, 0.285 mmol) were added, and the mixture was stirred at room temperature for 4 h. It was concentrated and the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (30 : 1, v/v) to give a cyano compound (209 mg, 95%). A solution of this cyano compound (209 mg, 0.271 mmol) in 2 ml of MeOH was treated with 40% methylamine in MeOH (0.117 ml, 1.36 mmol). The reaction mixture was stirred at room temperature for 16 h, then concentrated, and the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (30 : 1, v/v) to give **43** (165 mg, 86%). Softening point 154—156 °C. $[\alpha]_D^{25}$ −2.0° (c=1.018, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 2.60 (t, 2H, CH_2, J=6.6 Hz), 2.72 (s, 3H, $NHCH_3$), 3.24 (t, 2H, CH_2, J=6.6 Hz), 3.48 (s, 3H, N–CH_3), 3.63 (s, 2H, CH_2), 4.43 (s, 2H, CH_2), 5.35 (s, 1H, 3-H), 6.85 (s, 1H, thiazole-CH), 7.02—7.69 (m, 13H, Ar-H). *Anal.* Calcd for $C_{34}H_{36}N_{12}O_2S_2 \cdot 2.1H_2O$: C, 54.68; H, 5.43; N, 22.51; S, 8.59. Found: C, 54.61; H, 5.46; N, 22.43; S, 8.43. This method was also used to obtain other type III hybrid compounds (**44**, **45**, **46**), but not compound **42**. The softening points, the optical rotations, NMR, and analytical data were as follows.

(−)-(*R*)-1-[3-(*N'*-{4-[2-(*N*-Methyl-*N'*-aminosulfonylguanidino)ethylthiomethyl]thiazol-2-yl}guanidinomethyl)phenyl]-3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)urea (44) Softening point 155—157 °C. $[\alpha]_D^{25}$ −2.1° (c=1.013, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 2.65 (t, 2H, CH_2, J=6 Hz), 2.74 (s, 3H, $NHCH_3$), 3.30 (t, 2H, CH_2, J=6 Hz), 3.54 (s, 3H, N–CH_3), 3.65 (s, 2H, CH_2), 4.41 (s, 2H, CH_2), 5.36 (s, 1H, 3-H), 6.55 (s, 1H, thiazole-CH), 6.98—7.75 (m, 13H, Ar-H). *Anal.* Calcd for $C_{33}H_{38}N_{12}O_4S_3 \cdot 1.3H_2O$: C, 50.40; H, 5.20; N, 21.38; S, 12.23. Found: C, 50.43; H, 5.38; N, 21.12; S, 11.97.

(*R*)-1-[3-(*N'*-{4-[2-(1-Methylamino-2-nitrovinylamino)ethylthiomethyl]thiazol-2-yl}guanidinomethyl)phenyl]-3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)urea (45) Softening point 180—182 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.71 (t, 2H, CH_2, J=8 Hz), 2.79 (s, 3H, $NHCH_3$), 3.30 (t, 2H, CH_2, J=8 Hz), 3.50 (s, 3H, N–CH_3), 3.65 (s, 2H, CH_2), 4.42 (s, 2H, CH_2), 5.36 (s, 1H, 3-H), 6.51 (s, 1H, thiazole-CH), 7.00—7.75 (m, 13H, Ar-H). *Anal.* Calcd for $C_{34}H_{37}N_{11}O_4S_2$: C, 56.10; H, 5.12; N, 21.17; S, 8.81. Found: C, 56.23; H, 5.04; N, 21.29; S, 8.61.

(−)-(R)-1-[3-(*N'*-{4-[2-(3-Methylthioureido)ethylthiomethyl]thiazol-2-yl}guanidinomethyl)phenyl]-3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)urea (46) Softening point 148—150 °C. $[\alpha]_D^{25}$ −1.9° (c=1.001, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 2.64 (t, 2H, CH_2, J=8 Hz), 2.73 (s, 3H, $NHCH_3$), 3.38 (s, 3H, N–CH_3), 3.55—3.65 (m, 4H, $2CH_2$), 4.27 (s, 2H, CH_2), 5.42 (d, 1H, 3-H, J=7.8 Hz), 6.34 (s, 1H, thiazole-CH), 7.06—7.60 (m, 13H, Ar-H). *Anal.* Calcd for $C_{33}H_{36}N_{10}O_2S_3$: C, 56.54; H, 5.18; N, 19.99; S,13.73. Found: C, 56.44; H, 5.21; N, 20.03; S, 13.56.

Preparation of Intermediates used for the Synthesis of Hybrid Compounds. 1-(3-Hydroxybenzyl)piperidine-4-carboxylic Acid *tert*-Butyl Ester (3) The amine **2** (3.70 g, 20 mmol) was added to an ice-cooled solution of 3-hydroxybenzaldehyde (2.44 g, 20 mmol) in 20 ml of EtOH with stirring. Stirring was continued for 20 min at this temperature, then sodium borohydride ($NaBH_4$) (0.76 g, 20 mmol) was added and the mixture was stirred at ice-cooled temperature for 1 h. The temperature was gradually raised to room temperature and maintained for 16 h. After addition of aqueous ammonium chloride (NH_4Cl) solution, the reaction mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous sodium chloride (NaCl) solution, dried over sodium sulfate (Na_2SO_4) and concentrated. The residue was chromatographed on a silica gel column with *n*-hexane–EtOAc (1 : 1, v/v) to give **3** (1.52 g, 27%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (s, 9H, *tert*-Bu), 1.64—2.24 (m, 7H, $3.5CH_2$), 2.78—2.92 (m, 2H, CH_2), 3.45 (s, 2H, CH_2), 6.88—7.22 (m, 4H, Ar-H). Oxalate: mp 82—83 °C (acetone). *Anal.* Calcd for $C_{17}H_{25}NO_3$ $(CO_2H)_2 \cdot 1.0H_2O$: C, 57.13; H, 7.32; N, 3.51. Found: C, 56.86; H, 7.17; N, 3.54.

1-[3-(3-Aminopropoxy)benzyl]piperidine-4-carboxylic Acid *tert*-Butyl Ester (4) A solution of **3** (1.397 g, 5 mmol) in 5 ml of DMF was added dropwise to an ice-cooled suspension of sodium hydride (NaH) (60% oil dispersion, 0.12 g, 5 mmol) in 5 ml of DMF. The mixture was stirred for 1 h at room temperature, then a solution of the bromide (1.341 g, 5 mmol) in 5 ml of DMF was added dropwise and the whole was stirred for 4 h at room temperature. It was poured into ice-cooled water and extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl solution, dried over Na_2SO_4, and concentrated. The residue was chromatographed on a silica gel column with *n*-hexane–EtOAc (7 : 3, v/v) to give the desired intermediate (2.04 g, 87%). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (s, 9H, *tert*-Bu), 1.62—2.32 (m, 9H, $4.5CH_2$), 2.74—2.88 (m, 2H, CH_2), 3.41 (s, 2H, CH_2), 3.91 (t, 2H, CH_2, J=6.8 Hz),

4.03 (t, 2H, CH_2, J=5.8 Hz), 6.64—7.18 (m, 4H, Ar-H), 7.64—7.88 (m, 4H, Ar-H).

A mixture of the intermediate (0.467 g, 1 mmol) obtained above and hydrazine monohydrate (0.100 g, 2 mmol) in EtOH (10 ml) was refluxed for 2 h and then cooled. The mixture was diluted with ether, the precipitates were filtered off and the filtrate was concentrated *in vacuo*. The residue was chromatographed on a silica gel column with CH_2Cl_2–MeOH (9 : 1, v/v) to give the intermediate amine compound **4** (0.217 g, 64%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (s, 9H, *tert*-Bu), 1.64—2.30 (m, 9H, 4.5CH_2), 2.78—2.94 (m, 2H, CH_2), 3.04 (t, 2H, CH_2, J=6.8 Hz), 3.48 (s, 2H, CH_2), 3.90 (m, 2H, CH_2), 4.06 (t, 2H, CH_2, J=6.8 Hz), 6.72—7.24 (m, 4H, Ar-H). Oxalate: mp 102—104 °C (MeOH–acetone). *Anal.* Calcd for $C_{20}H_{32}N_2O_3 \cdot 2(CO_2H)_2 \cdot 1.0H_2O$: C, 52.74; H, 7.01; N, 5.13. Found: C, 53.02; H, 6.74; N, 5.31.

1-{3-[3-(2-Acetoxyacetylamino)propoxy]benzyl}piperidine-4-carboxylic Acid (5) Acetoxyacetyl chloride (0.406 g, 2.972 mmol) was added dropwise to an ice-cooled solution of the amine compound **4** (1.0 g, 2.972 mmol) and Et_3N (0.301 g, 2.972 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred for 10 min under ice-cooling, then washed with aqueous $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated. The residue was chromatographed on a silica gel column with EtOAc to give the desired intermediate (0.901 g, 69%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (s, 9H, *tert*-Bu), 2.15 (s, 3H, CH_3), 1.64—2.30 (m, 9H, 4.5CH_2), 2.76—2.92 (m, 2H, CH_2), 3.48 (s, 2H, CH_2), 3.55 (q, 2H, CH_2, J=6.4 Hz), 4.08 (t, 2H, CH_2, J=5.8 Hz), 4.57 (s, 2H, CH_2), 6.58—7.26 (m, 4H, Ar-H).

A mixture of the intermediate (0.436 mg, 1 mmol) obtained above and trifluoroacetic acid (1 ml) was stirred for 16 h at room temperature, then concentrated. The residue was chromatographed on a silica gel column with CH_2Cl_2–MeOH (7 : 3, v/v) to give **5** (0.294 g, 75%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 2.15 (s, 3H, CH_3), 1.82—2.68 (m, 9H, 4.5CH_2), 3.08—3.36 (m, 2H, CH_2), 3.47 (t, 2H, CH_2, J=6.2 Hz), 3.96 (s, 2H, CH_2), 3.99 (t, 2H, CH_2, J=5.8 Hz), 4.54 (s, 2H, CH_2), 6.92—7.35 (m, 4H, Ar-H).

(*R*)-1-[3-(2-Aminoethylthiomethyl)phenyl]-3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)urea (9) Compound **9** was prepared by a similar method to that used for compound **31**. Softening point 111—113 °C. ^{1}H-NMR (CD_3OD) δ: 2.70 (t, 2H, CH_2, J=7 Hz), 3.05 (t, 2H, CH_2, J=7 Hz), 3.61 (s, 3H, N–CH_3), 3.78 (s, 2H, CH_2), 5.65 (s, 1H, 3-H), 7.00—8.05 (m, 13H, Ar-H). *Anal.* Calcd for $C_{26}H_{27}N_5O_2S \cdot 1.0H_2O$: C, 63.52; H, 5.95; N, 14.25. Found: C, 63.41; H, 5.83; N, 14.16.

(*R*)-(2-Aminoethyl)carbamic Acid 3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzyl Ester (11) Compound **11** was prepared by the same method used for compounds **17** and **30**. Softening point 149—151 °C. ^{1}H-NMR (CD_3OD) δ: 2.85 (t, 2H, CH_2, J=6 Hz), 3.28 (t, 2H, CH_2, J=6 Hz), 3.51 (s, 3H, N–CH_3), 5.05 (s, 2H, CH_2), 5.37 (s, 1H, 3-H), 6.95—7.77 (m, 13H, Ar-H). *Anal.* Calcd for $C_{27}H_{28}N_6O_4 \cdot H_2O$: C, 62.53; H, 5.83; N, 16.21. Found: C, 62.27; H, 5.85; N, 16.02.

(−)-(*R*)-2-Amino-*N*-{3-[3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzyl}acetamide (13) Compound **13** was prepared by method A. Softening point 170—172 °C. $[\alpha]_D^{25}$ −26.0° (c=1.014, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 3.51 (s, 3H, N–CH_3), 3.69 (s, 2H, CH_2), 4.39 (s, 2H, CH_2), 5.37 (s, 1H, 3-H), 7.12—7.79 (m, 13H, Ar-H). *Anal.* Calcd for $C_{28}H_{27}F_3N_6O_5 \cdot 2.8H_2O$: C, 52.96; H, 5.18; N, 13.23; F, 8.98. Found: C, 52.84; H, 4.97; N, 12.99; F, 9.22.

{3-[3-(4-Isocyanatopiperidine-1-ylmethyl)phenoxy]propylcarbamoyl}methyl Acetate (16) A suspension of **5** (39 mg, 0.1 mmol) in acetone (1 ml) and H_2O (1 ml) was ice-cooled, then Et_3N (0.015 ml, 0.11 mmol) and ethyl chloroformate (0.012 ml, 0.13 mmol) were added in portions. The mixture was stirred for 5 min, then a solution of sodium azide (NaN_3) (10 mg, 0.15 mmol) in H_2O (1 ml) was added, and the whole was kept at 0 °C for 1 h, and extracted with CH_2Cl_2. The organic layer was washed with saline, dried over Na_2SO_4 and concentrated. The crude azide obtained as the residue was used in the next step without purification. IR ($CHCl_3$) cm^{-1}: 2258, 2132 (N_3).

A solution of this azide in 5 ml of benzene was refluxed for 30 min and the reaction mixture was concentrated *in vacuo*. The residue was used for the next step without purification. IR ($CHCl_3$) cm^{-1}: 2260 (NCO).

{3-[3-(4-Hydroxymethylpiperidin-1-ylmethyl)phenoxy]propylcarbamoyl}methyl Benzoate (19) Compound **19** was synthesized by method A. ^{1}H-NMR ($CDCl_3$) δ: 1.23—2.11 (m, 9H, 4CH_2+CH), 2.87—2.98 (m, 2H, CH_2), 3.48 (s, 2H, CH_2), 3.47—3.63 (m, 4H, 2CH_2), 4.08—4.15 (m, 2H, CH_2), 4.83 (s, 2H, CH_2), 6.87—8.08 (m, 9H, Ar-H). *Anal.* Calcd for $C_{25}H_{32}N_2O_5 \cdot 2.0H_2O$: C, 63.00; H, 7.61; N, 5,88. Found: C, 62.81; H, 7.33; N, 5.89.

{3-[3-(4-Azidomethylpiperidin-1-ylmethyl)phenoxy]propylcarbamoyl}methyl Benzoate (20) Compound **20** was prepared by the same method as used for compound **30**. IR ($CHCl_3$) cm^{-1}: 2098 (N_3). ^{1}H-NMR ($CDCl_3$) δ: 1.21—2.14 (m, 9H, 4CH_2+CH), 2.82—2.94 (m, 2H, CH_2), 3.13—3.20 (m, 2H, CH_2), 3.42 (s, 2H, CH_2), 3.53—3.65 (m, 2H, CH_2), 4.07 (m, 2H, CH_2), 4.83 (s, 2H, CH_2), 6.85—8.07 (m, 9H, Ar-H). *Anal.* Calcd for $C_{25}H_{31}N_5O_4 \cdot 0.6H_2O$: C, 63.03; H, 6.81; N, 14.70. Found: C, 63.10; H, 6.74; N, 14.48.

{3-[3-(4-Aminomethylpiperidin-1-ylmethyl)phenoxy]propylcarbamoyl}methyl Benzoate (21) Compound **21** was prepared by the same method as used for compound **30**. ^{1}H-NMR ($CDCl_3$) δ: 1.60—2.15 (m, 8H, 4CH_2), 2.34—2.64 (m, 2H, CH_2), 2.82—2.94 (m, 3H, CH_2+CH), 3.40 (s, 2H, CH_2), 3.52—3.63 (m, 2H, CH_2), 4.83 (s, 2H, CH_2), 6.83—8.11 (m, 9H, Ar-H).

(+)-(*R*)-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]phenyl}acetic Acid (22) Softening point 142—144 °C. $[\alpha]_D^{25}$ +65.0° (c=1.005, THF). ^{1}H-NMR (CD_3OD) δ: 3.50 (s, 3H, N–CH_3), 3.55 (s, 2H, CH_2), 5.37 (s, 1H, 3-H), 6.80—7.80 (m, 13H, Ar-H). *Anal.* Calcd for $C_{25}H_{22}N_4O_4 \cdot 2.5H_2O$: C, 61.59; H, 5.58; N, 11.50. Found: C, 61.93; H, 5.73; N, 11.27.

(−)-(*R*)-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzyloxycarbonylamino}acetic Acid (25) Softening point 143—145 °C. $[\alpha]_D^{25}$ −7.8° (c=1.010, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 3.45 (s, 3H, N–CH_3), 3.82 (d, 2H, CH_2, J=5.4 Hz), 5.08, 4.97 (ABq, 2H, CH_2, J=12.4 Hz, $\Delta\nu$=22.2 Hz), 5.57 (d, 1H, 3-H, J=8.2 Hz), 7.14—7.58 (m, 13H, Ar-H). *Anal.* Calcd for $C_{27}H_{25}N_5O_6 \cdot H_2O$: C, 60.78; H, 5.10; N, 13.13. Found: C, 60.81; H, 4.99; N, 12.91.

2-{3-[3-(3-Hydroxymethylpiperidin-1-ylmethyl)phenoxy]propyl}isoindole-1,3-dione (28) Compound **28** was prepared by the same method as used for compound **3**. ^{1}H-NMR ($CDCl_3$) δ: 1.50—2.20 (m, 9H, 4.5CH_2), 2.70—2.94 (m, 2H, CH_2), 3.44 (s, 2H, CH_2), 3.55—3.65 (m, 2H, CH_2), 3.91 (t, 2H, CH_2, J=7.0 Hz), 4.40 (t, 2H, CH_2, J=5.8 Hz), 6.64—7.22 (m, 4H, Ar-H), 7.68—7.90 (m, 4H, Ar-H).

{3-[3-(3-Hydroxymethylpiperidin-1-ylmethyl)phenoxy]propylcarbamoyl}methyl Acetate (29) Compound **29** was prepared by the same method as used for compound **4**. ^{1}H-NMR ($CDCl_3$) δ: 1.50—2.20 (m, 9H, 4.5CH_2), 2.15 (s, 3H, CH_3), 2.70—2.94 (m, 2H, CH_2), 3.44 (s, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.55—3.65 (m, 2H, CH_2), 3.91 (t, 2H, CH_2, J=7.0 Hz), 4.40 (t, 2H, CH_2, J=5.8 Hz), 6.72—7.34 (m, 4H, Ar-H).

{3-[3-(3-Aminomethylpiperidin-1-ylmethyl)phenoxy]propylcarbamoyl}methyl Acetate (30) Triphenylphosphine (Ph_3P) (157 mg, 0.6 mmol) and diethyl azodicarboxylate (DEAD) (105 mg, 0.6 mmol) were added to an ice-cooled solution of **29** (189 mg, 0.5 mmol) in 2 ml of THF. After 5 min, hydrazoic acid (HN_3) (70 mg/ml in benzene, 0.92 ml) was added, then the reaction mixture was kept at room temperature for 1 h, and concentrated *in vacuo*. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (85 : 15, v/v) to give the desired azide as an oil. IR ($CHCl_3$) cm^{-1}: 2100 (N_3).

This azide was dissolved in 2 ml of THF and Ph_3P (157 mg, 0.6 mmol) was added. The mixture was kept at room temperature for 16 h, then H_2O (0.6 ml) was added and the whole was refluxed for 30 min. It was concentrated *in vacuo*, and the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (7 : 3, v/v) to give **30** (62 mg, 33%). Softening point 82—84 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.50—3.10 (m, 11H, 5.5CH_2), 2.15 (s, 3H, CH_3), 3.44 (s, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.55—3.65 (m, 2H, CH_2), 3.91 (t, 2H, CH_2, J=7.0 Hz), 4.07 (t, 2H, CH_2, J=5.8 Hz), 6.70—7.20 (m, 4H, Ar-H).

(3-{3-[3-(2-Aminoethylthiomethyl)piperidin-1-ylmethyl]phenoxy}propylcarbamoyl)methyl Acetate (31) An solution of **29** (189 mg, 0.5 mmol) in 1 ml of THF was ice-cooled, then Ph_3P (157 mg, 0.6 mmol), DEAD (105 mg, 0.6 mmol), and a THF solution of *N*-Boc-thiol (124 mg, 0.6 mmol) were added in portions in the usual manner. The mixture was kept at room temperature for 2 h, then concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (85 : 15, v/v) to give the *N*-Boc compound (84 mg, 31%). This *N*-Boc compound (54 mg, 0.1 mmol) was treated with 1 ml of trifluoroacetic acid at room temperature for 4 h. The reaction mixture was concentrated *in vacuo*, and the residue was used for the next step without purification. ^{1}H-NMR ($CDCl_3$) δ: 1.48—3.13 (m, 11H, 5.5CH_2), 2.17 (s, 3H, CH_3), 3.44 (s, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.55—3.65 (m, 2H, CH_2), 3.80 (t, 2H,

CH_2, $J=7.0$ Hz), 3.91 (t, 2H, CH_2, $J=7.0$ Hz), 4.07 (t, 2H, CH_2, $J=5.8$ Hz), 4.12—4.33 (m, 2H, CH_2), 6.70—7.20 (m, 4H, Ar-H).

(−)-(*R*)-3-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)ureido]benzylthio}propionic Acid (32) $[\alpha]_D^{25}$ −1.2° ($c=0.511$, $CHCl_3$–0.1%EtOH). ^{1}H-NMR (CD_3OD) δ: 2.45—2.70 (m, 4H, $2CH_2$), 3.50 (s, 3H, N–CH_3), 3.70 (s, 2H, CH_2), 5.37 (s, 1H, 3-H), 6.95—7.80 (m, 13H, Ar-H). *Anal.* Calcd for $C_{27}H_{26}N_4O_4S \cdot H_2O$: C, 62.29; H, 5.42; N, 10.76; S, 6.16. Found: C, 61.99; H, 5.68; N, 10.46; S, 5.91.

1-[4-(2-Cyanoethylthiomethyl)thiazol-2-yl]-2-methylisothiourea Hydroiodide (36) ^{1}H-NMR (CD_3OD) δ: 2.73 (s, 3H, SCH_3), 2.74—2.79 (m, 4H, $2CH_2$), 3.89 (s, 2H, CH_2), 7.19 (s, 1H, CH).

{2-[2-(2-Methylisothioureido)thiazol-4-ylmethylthio]ethyl}carbamic Acid *tert*-Butyl Ester Hydroiodide (37) ^{1}H-NMR (CD_3OD) δ: 1.43 (s, 9H, *tert*-Bu), 2.57 (t, 2H, CH_2, $J=7$ Hz), 2.71 (s, 3H, SCH_3), 3.20 (t, 2H, CH_2, $J=7$ Hz), 3.79 (s, 2H, CH_2), 7.14 (s, 1H, thiazole-CH).

***N*-(Aminobenzyl)-*N'*-[4-(2-cyanoethylthiomethyl)thiazol-2-yl]guanidine (38)** A suspension of **36** (3.49 g, 8.72 mmol) and 3-aminobenzylamine (3.19 g, 26.1 mmol) in EtOH (50 ml) was refluxed for 20 h, then concentrated, and the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (15:1—10:1, v/v) to give **38** (2.21 g, 73%) as a powder. ^{1}H-NMR ($CDCl_3$) δ: 2.56—2.75 (m, 4H, $2CH_2$), 3.68 (s, 2H, CH_2), 4.33 (s, 2H, CH_2), 6.51 (s, 1H, CH), 6.60—6.75 (m, 3H, Ar-H), 7.05—7.12 (m, 1H, Ar-H).

(2-{2-[*N'*-(3-Aminobenzyl)guanidino]thiazol-4-ylmethylthio}ethyl)carbamic Acid *tert*-Butyl Ester (39) Compound **39** was prepared by the same method as used for compound **38**. ^{1}H-NMR (CD_3OD) δ: 1.43 (s, 9H, *tert*-Bu), 2.55 (t, 2H, CH_2, $J=7$ Hz), 3.18 (t, 2H, CH_2, $J=7$ Hz), 3.62 (s, 2H, CH_2), 4.46 (s, 2H, CH_2), 6.49 (s, 1H, thiazole-CH), 6.62—6.73 (m, 3H, Ar-H), 7.01—7.11 (m, 1H, Ar-H).

(+)-(*R*)-1-(3-{*N'*-[4-(2-Cyanoethylthiomethyl)thiazol-2-yl]guanidinomethyl}phenyl)-3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl)urea (40) Compound **38** (3.95 g, 10.2 mmol) was added to an ice-cooled solution of isocyanate (2.28 g, 7.83 mmol) in 20 ml of CH_2Cl_2, and the mixture was kept at room temperature for 2 h, then concentrated *in vacuo*. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (40:1—20:1, v/v). Fractions of the desired compound were collected and concentrated to give **40** (3.50 g, 70%) as a powder. $[\alpha]_D^{25}$ +7.9° ($c=1.004$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.47—2.75 (m, 4H, $2CH_2$), 3.40 (s, 3H, N–CH_3), 3.69 (s, 2H, CH_2), 4.39 (s, 2H, CH_2), 5.44 (d, 1H, 3-H, $J=7$ Hz), 6.50 (s, 1H, CH), 6.86 (d, 1H, NH, $J=9$ Hz), 7.10—7.59 (m, 13H, Ar-H).

(*R*)-{2-[2-(*N'*-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-ylureido]benzyl}guanidino)thiazol-4-ylmethylthio]ethyl}carbamic Acid *tert*-Butyl Ester (41) Compound **41** was prepared by the same method as used for compound **40**. ^{1}H-NMR (CD_3OD) δ: 1.41 (s, 9H, *tert*-Bu), 2.54 (t, 2H, CH_2, $J=7$ Hz), 3.17 (t, 2H, CH_2, $J=7$ Hz), 3.49 (s, 3H, N–CH_3), 3.61 (s, 2H, CH_2), 4.41 (s, 2H, CH_2), 5.36 (s, 1H, 3-H), 6.49 (s, 1H, thiazole-CH), 7.01—7.70 (m, 13H, Ar-H).

Bioassay Procedures

***In Vitro* Experiments** Binding assays for 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: A well established procedure was employed.[12]

***In Vivo* Experiments** Gastric Acid Secretion-Inhibitory Activity: Antisecretory activities were studied using pylorus ligation preparations.[13] Under ether anesthesia, the abdomen was incised, the pylorus ligated, histamine 2HCl (3 mg/kg, s.c.) injected, and the animal killed 2 h later. The gastric contents were collected and their volume and acidity were determined. Acidity was determined by automatic titration of the gastric juice with 0.1 N NaOH to pH 7.0 (Autoburette). Titratable acid output was expressed as μeq/2 h. Test compound or vehicle alone was given *p.o.* at 10 mg/kg 1 h before ligating the pylorus.

Acknowledgments We thank Drs. M. Narisada (former-director of the laboratories), T. Yoshida, S. Kamata, and M. Doteuchi for their generous support and suggestions throughout the course of this work. We also thank Messrs. K. Otani, F. Hirose, and G. Kato for the binding assays.

References and Notes

1) *a*) "Integrated Handbook of Internal Medicine," Vol. 42, ed. by Yamamura Y., Yoshitoshi K., Nakayama Syoten, Tokyo, 1991, pp. 26—40; Mitsuhashi T., Yokoyama Y., Saigenji K., *Rinsho Kagaku*, **26**, 43—49 (1990). For acid rebound, see *b*) Nwokolo C. U., Smith J. T. L., Sawyerr A. M., Pounder R. E., *Gut*, **32**, 1455—1460 (1991); Fullarton G. M., Macdonald A., McColl K. E. L., *Aliment. Pharmacol. Ther.*, **5**, 391—398, (1991); Fullarton G. M., McLauchlan G., Macdonald A., Crean G. P., McColl K. E. L., *Gut*, **30**, 449—454 (1989).
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.*, **6**, 1421—1426 (1996).
3) Kawanishi Y., Ishihara S., Tsushima T., Seno K., Miyagoshi M., Hagishita S., Ishikawa M., Shima N., Shimamura M., Ishihara Y., *Bioorg. Med. Chem. Lett.*, **6**, 1427—1430 (1996).
4) *a*) Takagi T., Takeda M., Maeno H., *Arch. Int. Pharmacodyn. Ther.*, **256**, 49—58 (1982); *b*) Takeda M., Takagi T., Yashima Y., Maeno H., *Arzneim.-Forsch.*, **32**, 734—737 (1982); *c*) Yanagisawa I., Hirata Y., Ishii Y., *J. Med. Chem.*, **27**, 849—857 (1984); *d*) *Idem*, *ibid.*, **30**, 1787—1793 (1987).
5) Tarutani M., Sakuma H., Shiratsuchi K., Mieda M., *Arzneim.-Forsch.*, **35**, 703—706 (1985).
6) 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.*, **32**, 13—16 (1989).
7) Ghosh M. N., Schild H. O., *Br. J. Pharmacol.*, **13**, 54—61 (1958).
8) Grossman M. I., Konturek S. J., *Gastroenterology*, **66**, 517—521 (1974).
9) Bock M. G., DiPardo R. M., Evans B. E., Rittle K. E., Veber D. F., Freidinger R. M., Hirshfield J., Springer J. P., *J. Org. Chem.*, **52**, 3232—3239 (1987).
10) Chang R. S. L., Lotti V. J., Keegan M. E., Kunkel K. A., *Biochem. Biophys. Res. Commun.*, **134**, 895—899 (1986).
11) Saito A., Goldfine I. D., Williams J. A., *J. Neurochem.*, **37**, 483—490 (1981).
12) Black J. W., Duncan W. A. M., Durant C. J., Ganellin C. R., Parsons E. M., *Nature* (London), **236**, 385—390 (1972).
13) Rat pylorus ligation method: Shay H., Komarov S. A., Fels S. S., Meranze D., Gruenstein M., Siplet H., *Gastroenterology*, **5**, 43—61 (1945).