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Synthesis and Structure–Activity Relationships of Dual Histamine H_2 and Gastrin Receptor Antagonists with Noncyclic Gastrin Receptor Antagonistic Moieties

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Abstract—In order to study structure–activity relationships of dual histamine H_2 and gastrin receptor antagonists as well as to improve their low oral absorbability, their prototype benzodiazepine gastrin receptor antagonistic moieties were altered to a conformationally flexible noncyclic dipeptide equivalent. This skeletal modification significantly potentiated the binding affinity of hybrid compounds for the histamine H_2 receptor, whereas their affinity for the gastrin receptor and receptor selectivity over the CCK-A receptor varied widely with the substituents on the gastrin moiety. Among them, {3-[3-(3-piperidin-1-ylmethylphenoxy)-propylcarbamoyl]propyl}carbamic acid 3-[3-({(3-methoxyphenyl)[(methylphenylcarbamoyl)methyl]carbamoyl}methyl)ureido]-benzyl ester (**7a**) showed the highest dual histamine H_2 and gastrin receptor antagonistic activities. It also displayed distinct gastric acid antisecretory activity in vivo for two assays, namely, Schild's rat method by id administration and the rat pylorus ligation method by oral administration. With the latter case, dose–response relationships were observed for the first time, suggesting its substantially improved oral absorbability. However, **7a** did not display distinct in vivo gastric acid antisecretory activity for the assay with Heidenhain pouch dogs. © 1997 Elsevier Science Ltd.

Introduction

In previous papers[1–5] we reported on the dual histamine H_2 and gastrin receptor antagonists (dual H_2 and GA) and the design, synthesis, and structure–activity relationships of various joint-type hybrid molecules composed of two pharmacophore moieties taken from known histamine H_2 and gastrin receptor antagonists (H_2A and GA). Among these compounds, **1**[2] displayed the highest in vitro dual H_2 and GA activities. However, preliminary evaluation of its in vivo gastric acid antisecretory activity disclosed a low oral absorbability of this compound. In order to study structure–activity relationships of dual H_2 and GA as well as to improve their low oral absorbability, we have tried various types of chemical modifications to date.[1–5] In our preceding paper, we examined the displacement of the prototype benzodiazepine GA moiety with another GA moiety such as benzazepine, benzoxazepine, or benzothiazepine[5] as we suspected that the rigid benzodiazepine structure involved in all previous hybrid compounds might itself be responsible for their low oral absorbability, since many benzodiazepine analogues of L-365,260[6] showed relatively low oral absorbability, particularly for higher classes of animals. Nevertheless, our results have not been fruitful. In this paper, we tried to displace the prototype benzodiazepine GA pharmacophore moiety with a noncyclic dipeptide type taken

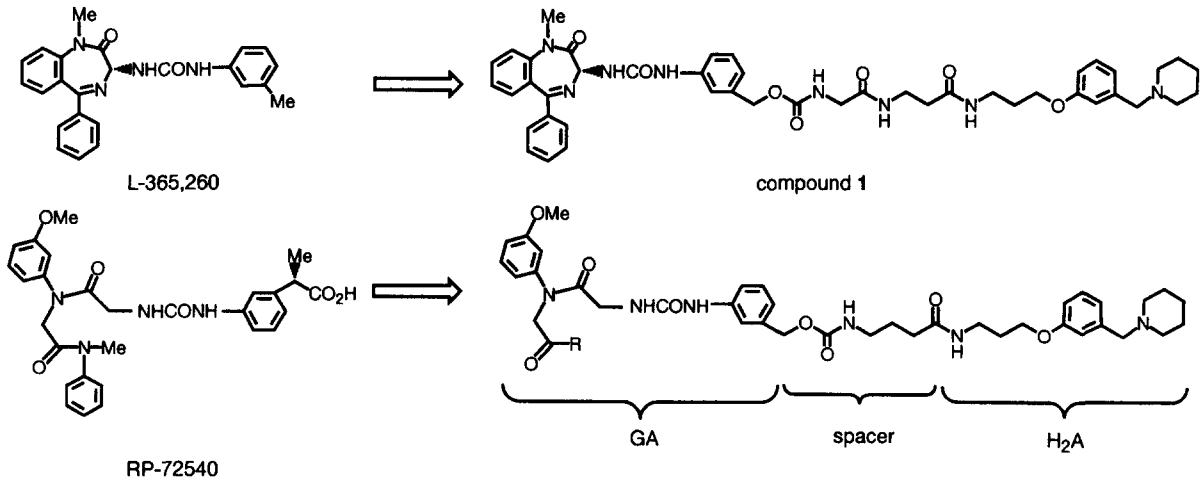


Figure 1.

from RP-72540[7] which was recently reported by the Rhône–Poulenc Rorer group as a novel noncyclic type of potent GA. We expected that this structural modification would substantially enhance oral absorbability of dual antagonists due to their increased conformational flexibility and/or somewhat diminished hydrophobicity. In this paper we report the synthesis and biological evaluation of hybrid molecules with flexible open-chain GA pharmacophore moieties instead of structurally rigid benzodiazepine GA ones.

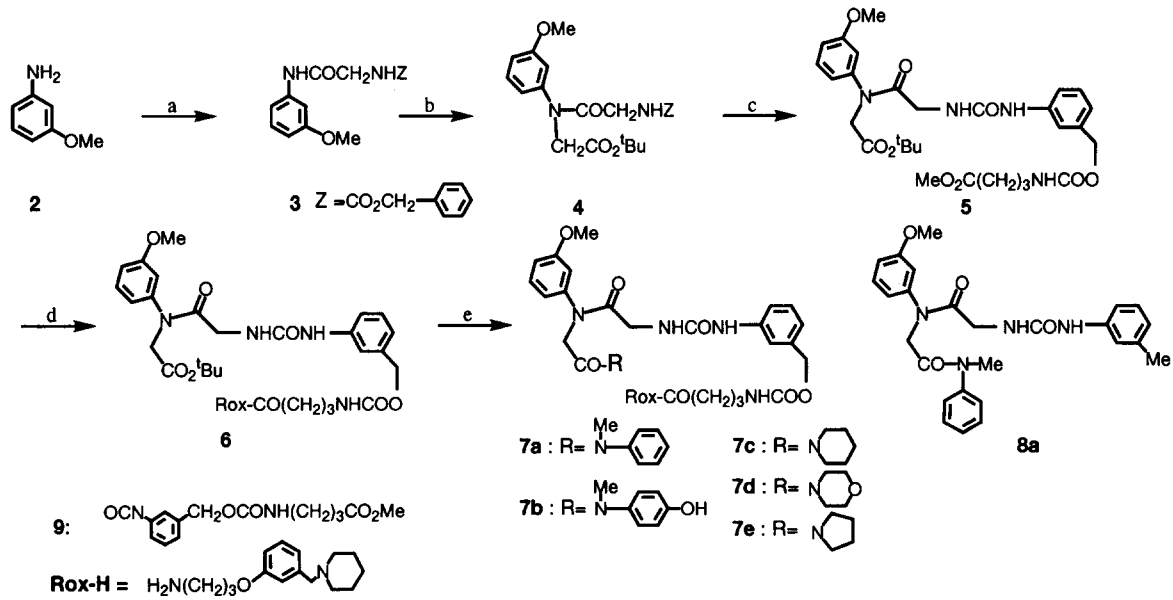
Chemistry

The molecular structures and synthetic schemes of the hybrid compounds are summarized in Scheme 1. As illustrated in Figure 1, our strategy for chemical modification is to use a basic dipeptide moiety taken from a potent GA, RP-72540, as a GA moiety and to construct hybrid molecules by connecting it with an H_2A pharmacophore using an appropriate spacer. We also attempt to change the terminal amino acid residue (R) of the GA moiety from *N*-methylaniline, probably an optimized R group in the discovery of RP-72540 though, to some others to study their pharmacological effects on the dual activities of our hybrid molecules. The synthetic details of these hybrid molecules are as follows.

The commercially available starting material, 3-anisidine **2**, was first treated with *N*-*Z*-glycine in the presence of dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), and triethylamine (Et_3N) under usual coupling conditions, to give amide **3**. It was sequentially treated with sodium hydride (NaH) and with *tert*-butyl bromoacetate to produce the *N*-alkylated product **4**. After removal of the benzyloxycarbonyl amino-protecting group by catalytic hydrogenation, the resultant amine was coupled with isocyanate **9** to give the urea compound **5**. Ester hydrolysis of **5** in aqueous potassium carbonate (K_2CO_3) followed by coupling the resultant free acid with an amine derivative of roxatidine Rox-H using coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSCI) produced an ester-type hybrid compound **6**. Other amide-type hybrid compounds **7a–7e** were derived from **6** by treatment with trifluoroacetic acid followed by coupling with various secondary amines as described above. This modification was an attempt to vary the hydrophilicity of these hybrid molecules by incorporating heterocyclic amine residues of different degrees of hydrophilicity.

Molecular modeling

In order to view the conformational similarity at the receptor-bound state of L-365,260 and the GA part of the hybrid compound **7a**, namely, **8a**, these two molecules were superimposed so as to attain their optimal fit. Figure 2 displays two types of the best fit conformations of these two molecules and obviously indicates that both *N*-methylanilino (as an R residue in Fig. 1) and *m*-methoxyphenyl groups of **8a** correspond to the *N*-methylanilino and C_5 phenyl group of L-365,260, respectively. Two other features are also deduced, namely, that three phenyl groups involved in L-365,260 and **8a** can be laid over each other well, and two carbonyl groups, one locating at the benzodiazepine C_2 position and the other attached to the *N*-methylaniline group, closely match each other, possibly suggesting their similar contribution to binding with the gastrin receptor through hydrogen bonding.



a: 1) $ZNHCH_2CO_2H$, HOBt, Et_3N, DCC b: NaH, $BrCH_2CO_2{}^tBu$ c: 1) Pd-C/H_2 2) **9**, Et_3N d: 1) K_2CO_3, aq.MeOH 2) **Rox-H**, HOBt, Et_3N, WSCI e: 1) CF_3CO_2H, anisole 2) various amines, HOBt, Et_3N, DCC

Scheme 1.

Biological Results and Discussion

The in vitro biological activities,[8,9,10] calculated hydrophobic parameter *C*log*P* values, and chemical structures of these hybrid compounds are summarized in Table 1. The *C*log*P* values were calculated using an appropriate computer program.[11]

As shown in Table 1, the *C*log*P* values of these noncyclic dipeptide-type hybrid compounds range from 3.567 to 4.977, a substantial drop from the 5.740 of the potent benzodiazepine-type hybrid compound **1**. This finding suggests a substantial enhancement of molecular hydrophilicity between previously reported cyclic benzodiazepine compounds and these structurally flexible noncyclic compounds. With this in mind, we discuss here the SAR of these noncyclic dual H_2 and GA. The most significant finding is that the pA_2 values of these hybrid compounds, which range from 6.7 to 7.6, markedly increase after displacement of the cyclic benzodiazepine moiety with a noncyclic GA moiety. Compound **7a** showed the highest recorded pA_2 value (7.6) of all the dual antagonists we have synthesized up to now, exceeding even the 7.3 of famotidine, the most potent H_2A on the market.

The GA activity of these hybrid compounds varied markedly with substituents at the GA terminal, with IC_{50} values ranging widely from 21 to 1200 nM. Compounds **7a** and **7c** with higher *C*log*P* values obviously showed higher GA activities than compounds **7d** and **7e** with lower *C*log*P* values. Among the amide-type compounds **7a–7e**, there appears to exist a rather simple correlation between their *C*log*P* values and their IC_{50} values: $\log IC_{50} = -1.290 \times (C\log P) + 7.916$ ($n = 5$, $r = -0.889$). Although the number of compounds involved in this analysis is quite small, from this simple correlation we made the following deduction. Since the calculated differences in the *C*log*P* values of these molecules solely reflect the differences in the *C*log*P* values of the R substituents, which were revealed to correspond to the *N*-methylaminobenzo moiety involved in the benzodiazepine skeleton of L-365,260 by our modeling in Figure 2, the observed pharmacological consequence of the chemical modification of R appears to accord highly with the experimentally well-proven importance of the *N*-methylaminobenzo moiety of L-365,260 as a hydrophobic GA pharmacophore.

Interestingly, **7a** showed the highest GA activity, IC_{50} = 21 nM, as well as a gastrin receptor selectivity 86 times that of the CCK-A receptor, though not as high as that of compound **1**. Surprisingly, the introduction of a hydroxyl group into the para position of the benzene ring of compound **7a** drastically abolished not only its GA activity but also its GA versus CCK-A receptor selectivity as shown by compound **7b**. Of the compounds bearing secondary cyclic amine substituents, **7c–7e**, only the piperidine compound **7c** showed a high GA activity of 73 nM. Though having the largest *C*log*P* value, the ester compound **6** completely lost both the GA activity and the gastrin versus CCK-A receptor selectivity.

We decided to focus on compound **7a**, as it showed a potent H_2A and GA activity as revealed by its pA_2 value of 7.6 and IC_{50} = 21 nM, respectively. Furthermore, its GA versus CCK-A receptor selectivity was substantially maintained. We expected compound **7a** to show significant improvement in oral absorbability as a consequence of increased conformational flexibility and its somewhat decreased high hydrophobicity. The

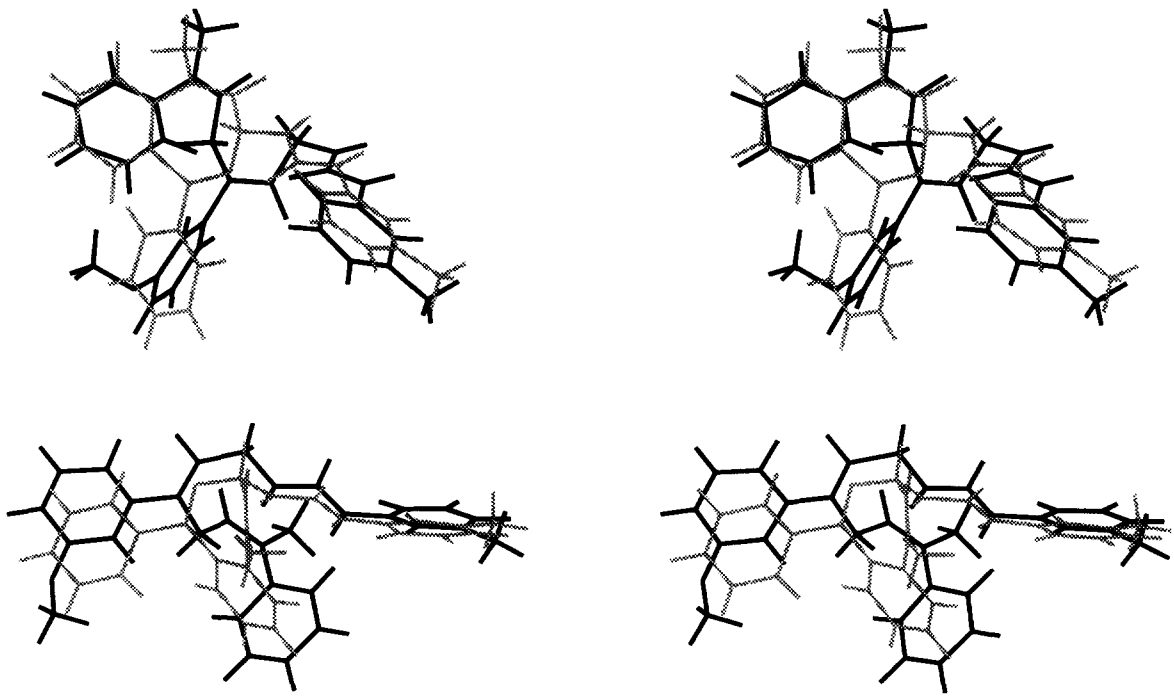
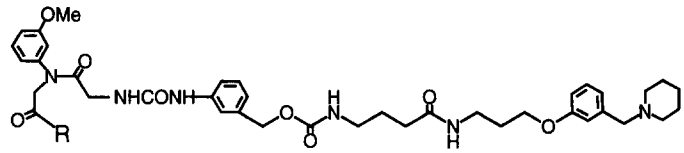
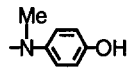


Figure 2. Two overlay models of L-365,260 (shaded) and GA moiety **8a** (solid) of compound **7a**.

Table 1. In vitro biological activities of hybrid compounds



No.	R	ClogP	Receptors IC_{50} (nM) Gastrin	CCK-B	CCK-A	Ratio Gastrin:CCK-A	pA_2 Histamine
6	–O^{t}Bu	4.977	230	435	220	1	6.9
7a		4.971	21	380	1800	86	7.6
7b		4.304	940	>1000	4000	4	6.8
7c		4.533	73	>1000	1070	15	7.1
7d		3.567	1200	>1000	2200	2	6.7
7e		3.974	640	>1000	720	1	7.0
1		5.740	19	103	8200	432	6.8
Famotidine							7.3
Roxatidine acetate							7.2
L-365,260			4	29	11,100	2775	

in vivo gastric acid antisecretory activity of **7a** was evaluated by two different methods, namely Schild's rat method[12] for id and iv administration and rat pylorus ligation method[13] for oral administration. When **7a** was administered to Schild's rat by an iv route, it strongly inhibited gastric acid secretion by 75% at a 1 mg kg^{-1} dose. However, as compound **1** was found to inhibit it by 91% under the same conditions, **7a** showed slightly lower gastric acid antisecretory potency in vivo than **1**, contrary to our expectation from its high pA_2 value (see Fig. 3). When **7a** was administered to Schild's rat by an id route, it inhibited acid secretion by 73% at a 30 mg kg^{-1} dose, which was the same as that of compound **1** (see Fig. 3).

Evaluation of oral in vivo gastric acid antisecretory activity by the rat pylorus ligation method for a 10 mg kg^{-1} dose revealed a slightly higher inhibitory activity for **7a**, 63%, than that of compound **1**, 58%, suggesting only marginal, if any, improvement in oral absorbability (Fig. 4). However, compound **7a** showed a dose–response relationship in this method for the first time, though in a narrow concentration range (3–20 mg kg^{-1}).

Encouraged by this finding, we tested compound **7a** for gastric acid antisecretory activity with the canine Heidenhain pouch method.[14] However, only marginal inhibition was found of histamine-stimulated gastric acid secretion (maximum 23.5% inhibition at 10 mg kg^{-1} po).

Conclusion

In this study, we synthesized a new type of dual H_2 and GA with flexible open-chain GA pharmacophore moieties instead of structurally rigid benzodiazepine ones. Compound **7a** displayed the highest record of the dual H_2 and GA activities in vitro as well as the dose–response relationships for the first time in the in vivo assay for oral gastric acid antisecretory activity by the rat pylorus ligation method. However, **7a** did not display substantial in vivo gastric acid antisecretory activity for the assay with Heidenhain pouch dogs, indicating its still poor oral absorbability, particularly for higher classes of animals. Other pharmacokinetic approaches including the use of various vehicles as well as other administration routes remain to be studied to improve the oral absorbability of these unique hybrid compounds with dual H_2A and GA activities.

Experimental

Chemistry

All softening points were determined on a Yanagimoto micromelting point apparatus and were not corrected. The ^{1}H 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. Column chromatography was performed on Merck Silica gel 60 (230–400 or 70–230 mesh).

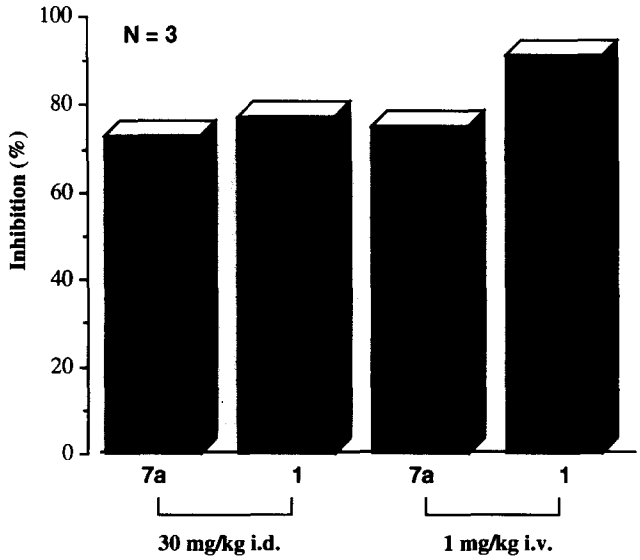


Figure 3. Effects of **7a** and **1** on histamine-stimulated gastric acid secretion in Schild's rats.

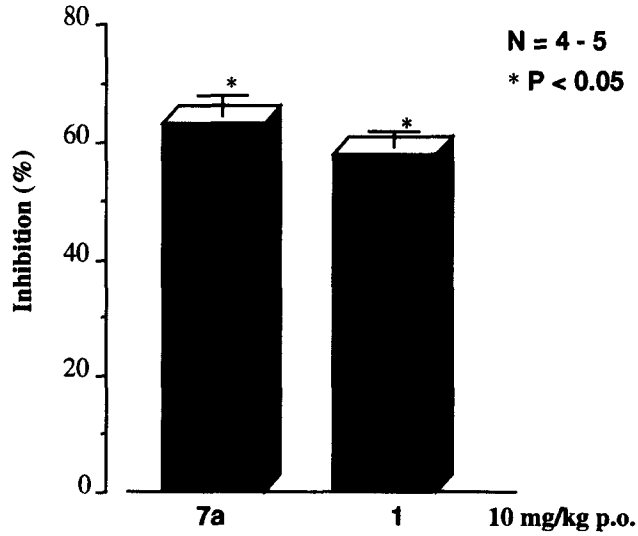


Figure 4. Effects of **7a** and **1** on histamine-stimulated gastric acid secretion in pylorus-ligated rats.

Hybrid compounds

[(3-Methoxyphenyl)-({3-[3-({3-[3-(3-piperidin-1-ylmethylphenoxy)propylcarbamoyl]propyl}carbamoyloxymethyl)-phenyl]ureido}acetyl)amino]acetic acid *tert*-butyl ester (6). A solution of potassium carbonate (K_2CO_3) (5.66 g, 40.8 mmol) in 24 ml of water (H_2O) was added with stirring into an ice-cooled solution of **5** (6.0 g, 10.2 mmol) in 66 ml of methanol (MeOH). After stirring for 16 h at room temperature, this reaction mixture was neutralized with 1 N hydrochloric acid (HCl) and concentrated in vacuo. The residue was extracted with ethyl acetate (EtOAc). The organic layer was washed with satd sodium chloride (NaCl), dried over magnesium sulfate ($MgSO_4$), and concentrated. The residue (5.56 g) was used for the next step without further purification.

1-Hydroxybenzotriazol (HOBt) (1.18 g, 8.73 mmol), triethylamine (Et_3N) (3.65 ml, 26.2 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSCI) (2.18 g, 11.4 mmol) were added stepwise under ice cooling into a well-stirred 50 ml quantity of dimethylformamide (DMF) solution containing the amine derivative of roxatidine Rox-H (2.82 g, 11.4 mmol) and the carboxylic acid derivative obtained above (5.0 g, 8.73 mmol). The reaction mixture was stirred 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 $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on a silica gel colomn using a solvent mixture $CHCl_3$–MeOH (10:1, v/v) as an eluent to give **6** (5.39 g, 77%). Softening point 54–56 °C. ^1H NMR ($CDCl_3$) δ: 1.35–1.47 (m, 11H, CH_2+*tert*-Bu), 1.51–1.64 (m, 4H, $2CH_2$), 1.74–2.00 (m, 4H, $2CH_2$), 2.20 (t, 2H, CH_2, J = 6 Hz), 2.30–2.50 (m, 4H, $2CH_2$), 3.12–3.25 (m, 2H, CH_2), 3.32–3.40 (m, 2H, CH_2), 3.45 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.89 (d, 2H, CH_2, J = 5 Hz), 3.97 (t, 2H, CH_2, J = 6 Hz), 4.24 (s, 2H, CH_2), 4.94 (s, 2H, CH_2), 6.72–7.37 (m, 12H, Ar-H). Anal. calcd for $C_{42}H_{58}N_6O_9 \cdot 0.8H_2O$: C, 63.18; H, 7.35; N, 10.28. Found: C, 63.11; H, 7.39; N, 10.21.

{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-propyl}carbamic acid 3-[3-({(3-methoxyphenyl)-[(methylphenylcarbamoyl)methyl]carbamoyl}methyl)-ureido]benzyl ester (7a). Trifluoroacetic acid (24.3 ml) and anisole (2.28 ml) were added with stirring into an ice-cooled solution of **6** (8.44 g, 10.51 mmol) in 124 ml of dichloromethane (CH_2Cl_2). After stirring for 16 h at room temperature, the reaction mixture was concentrated, toluene was added to the residue, and the solution was again concentrated. This operation was repeated twice. Ether was added to the residue and then decanted off. The residue was concentrated in vacuo. The residue (8.60 g) was used for the next step without further purification.

HOBt (1.14 g, 8.4 mmol), Et_3N (3.5 ml, 25.2 mmol), and 1,3-dicyclohexylcarbodiimide (DCC) (2.26 g, 11.0 mmol) were added stepwise under ice cooling into a well-stirred 50 ml quantity of DMF solution containing *N*-methylaniline (1.18 ml, 10.9 mmol) and the carboxylic acid derivative obtained above (7.24 g, 8.4 mmol). The reaction mixture was stirred at room temperature for 16 h, and concentrated under vacuum. 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 $CHCl_3$–MeOH (20:1, v/v) as an eluent to give **7a** (4.5 g, 64%). Softening point 67–69 °C. ^1H NMR ($CDCl_3$) δ: 1.37–2.03 (m, 10H, $5CH_2$), 2.18–2.30 (m, 2H, CH_2), 2.40–2.68 (m, 4H, $2CH_2$), 3.17–3.28 (m, 2H, CH_2), 3.22 (s, 3H, N-CH_3), 3.29–3.45 (m, 2H, CH_2), 3.53–3.68 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.84–4.10 (m, 4H, $2CH_2$), 4.09 (s, 2H, CH_2), 4.97 (s, 2H, CH_2), 6.67–7.74 (m, 17H, Ar-H). Anal. calcd for $C_{46}H_{57}N_7O_8 \cdot 1.4H_2O$: C, 64.15; H, 7.00; N, 11.39. Found: C, 64.20; H, 7.13; N, 11.10. With this method, other

hybrid compounds (**7b**, **7c**, **7d**, and **7e**) were obtained. Their softening points, NMR, and analytical data were as follows.

{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-propyl}carbamic acid 3-(3-{[{[(4-hydroxyphenyl)-methylcarbamoyl]methyl}-(3-methoxyphenyl)carbamoyl]-methyl}ureido)benzyl ester (7b). Softening point 77–79 °C. ^{1}H NMR ($CDCl_3$) δ: 1.34–1.52 (m, 2H, CH_2), 1.52–1.70 (m, 4H, $2CH_2$), 1.72–1.98 (m, 4H, $2CH_2$), 2.19 (t, 2H, CH_2, J = 6 Hz), 2.38–2.60 (m, 4H, $2CH_2$), 2.79 (s, 3H, N-CH_3), 3.08–3.25 (m, 2H, CH_2), 3.26–3.42 (m, 2H, CH_2), 3.53 (s, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.84–4.00 (m, 4H, $2CH_2$), 4.53 (s, 2H, CH_2), 4.96 (s, 2H, CH_2), 6.48–7.36 (m, 16H, Ar-H). Anal. calcd for $C_{46}H_{57}N_7O_9 \cdot 1.6H_2O$: C, 62.72; H, 6.89; N, 11.13. Found: C, 62.75; H, 6.89; N, 11.25.

{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-propyl}carbamic acid 3-(3-{[(3-methoxyphenyl)-(2-oxo-2-piperidin-1-ylethyl)carbamoyl]methyl}ureido)-benzyl ester (7c). Softening point 73–75 °C. ^{1}H NMR ($CDCl_3$) δ: 1.33–1.62 (m, 12H, $6CH_2$), 1.72–2.00 (m, 4H, $2CH_2$), 2.20 (t, 2H, CH_2, J = 7 Hz), 2.30–2.46 (m, 4H, $2CH_2$), 3.10–3.44 (m, 8H, $4CH_2$), 3.45 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.89–4.02 (m, 4H, $2CH_2$), 4.42 (s, 2H, CH_2), 4.98 (s, 2H, CH_2), 6.72–7.34 (m, 12H, Ar-H). Anal. calcd for $C_{44}H_{59}N_7O_8 \cdot 0.9H_2O$: C, 63.65; H, 7.38; N, 11.81. Found: C, 63.62; H, 7.37; N, 11.85.

{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-propyl}carbamic acid 3-(3-{[(3-methoxyphenyl)-(2-morpholin-4-yl-2-oxoethyl)carbamoyl]methyl}ureido)-benzyl ester (7d). Softening point 79–81 °C. ^{1}H NMR (CD_3OD) δ: 1.36–1.68 (m, 6H, $3CH_2$), 1.72–2.02 (m, 4H, $2CH_2$), 2.21 (t, 2H, CH_2, J = 7.6 Hz), 2.36–2.52 (m, 4H, $2CH_2$), 3.12–3.24 (m, 2H, CH_2), 3.26–3.42 (m, 4H, $2CH_2$), 3.47 (s, 2H, CH_2), 3.48–3.63 (m, 4H, $2CH_2$), 3.79 (s, 3H, OCH_3), 3.89–4.02 (m, 4H, $2CH_2$), 4.42 (s, 2H, CH_2), 4.95 (s, 2H, CH_2), 6.73–7.35 (m, 12H, Ar-H). Anal. calcd for $C_{43}H_{57}N_7O_9 \cdot 1.2H_2O$: C, 61.66; H, 7.14; N, 11.71. Found: C, 61.66; H, 7.12; N, 11.73.

{3-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-propyl}carbamic acid 3-(3-{[(3-methoxyphenyl)-(2-oxo-2-pyrrolidin-1-ylethyl)carbamoyl]methyl}ureido)-benzyl ester (7e). Softening point 72–74 °C. ^{1}H NMR (CD_3OD) δ: 1.37–1.62 (m, 6H, $3CH_2$), 1.70–2.00 (m, 8H, $4CH_2$), 2.21 (t, 2H, CH_2, J = 6 Hz), 2.30–2.48 (m, 4H, $2CH_2$), 3.12–3.25 (m, 2H, CH_2), 3.28–3.40 (m, 6H, $3CH_2$), 3.44 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.89–4.00 (m, 4H, $2CH_2$), 4.34 (s, 2H, CH_2), 5.00 (s, 2H, CH_2), 6.72–7.36 (m, 12H, Ar-H). Anal. calcd for $C_{43}H_{57}N_7O_8 \cdot 1.1H_2O$: C, 63.01; H, 7.28; N, 11.96. Found: C, 62.97; H, 7.30; N, 12.04.

Preparation of intermediates used for the synthesis of hybrid compounds

[(3-Methoxyphenylcarbamoyl)methyl]carbamic acid benzyl ester (3). HOBt (6.18 mg, 45.7 mmol), Et_3N (12.8 ml, 91.4 mmol), and DCC (12.26 g, 59.4 mmol) were added stepwise under ice cooling into a well-stirred solution containing 3-methoxyaniline **2** (7.32 g, 59.4 mmol) and benzyloxycarbonylamino-acetic acid (9.54 g, 45.7 mmol) in 100 ml of DMF. The reaction mixture was stirred at room temperature for 16 h, and concentrated under vacuum. EtOAc was added to the residue and the insoluble solid was removed by filtration. The organic phase was 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 silica gel using $CHCl_3$–acetone (10:1, v/v) as an eluent to give **3** (11.68 g, 81 %). ^{1}H NMR ($CDCl_3$) δ: 3.78 (s, 3H, OCH_3), 4.00 (d, 2H, CH_2, J = 6 Hz), 5.16 (s, 2H, CH_2), 6.65–7.35 (m, 9H, Ar-H).

[Benzyloxycarbonylaminoacetyl-(3-methoxyphenyl)-amino]acetic acid *tert*-butyl ester (4). Sodium hydride 60% dispersion in mineral oil (NaH) (1.65 g, 41.3 mmol) was added to an ice-cooled solution of **3** (10.0 g, 31.8 mmol) in 100 ml of THF, then after 5 min, bromoacetic acid *tert*-butyl ester (7.2 ml, 44.6 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. After the addition of water to the reaction mixture, the products were extracted with $CHCl_3$, washed with 1 N HCl and H_2O, dried over $MgSO_4$, and concentrated under vacuum. The residue was chromatographed on a silica gel column using toluene–EtOAc (10:1, v/v) as an eluent to give **4** (11.1 g, 81%). ^{1}H NMR ($CDCl_3$) δ: 1.46 (s, 9H, *tert*-Bu), 3.77–3.83 (m, 5H, OCH_3+CH_2), 4.24 (s, 2H, CH_2), 5.07 (s, 2H, CH_2) 6.89–7.33 (m, 9H, Ar-H).

4-[3-(3-{[*tert*-Butoxycarbonylmethyl-(3-methoxyphenyl)-carbamoyl]methyl}ureido)benzyloxycarbomylamino]-butyric acid methyl ester (5). Compound **4** (11.1 g, 25.8 mmol) was dissolved in 50 ml of MeOH and hydrogen gas was introduced under normal pressure in the presence of 10% palladium-on-charcoal (Pd–C) catalyst (2.0 g) at room temperature for 1 h. After filtrating off the catalyst through a celite layer, the filtrate was concentrated in vacuo to give crude amine (7.6 g). Et_3N (3.60 ml, 25.8 mmol) and isocyanate **9** (8.3 g, 28.4 mmol) were added to an ice-cooled solution of this amine (7.6 g, 25.8 mmol) in 70 ml of CH_2Cl_2, and the reaction mixture was stirred at the same temperature for 1 h. The reaction mixture was concentrated, and the residue was chromatographed on a silica gel column using $CHCl_3$–acetone (20:1, v/v) as an eluent to give **5** (14.86 g, 98%). ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H, *tert*-Bu), 3.68–3.72 (m, 2H, CH_2), 3.82 (s, 3H, OCH_3), 4.29 (s, 2H, CH_2) 6.89–7.38 (m, 4H, Ar-H).

Molecular modeling

The molecular models of the two GAs used were constructed within the molecular modeling software package SYBYL 6.30, using molecular fragments and standard bond lengths and angles from the SYBYL structural library. Each structure was optimized by means of the molecular mechanics minimizer MAX-

IMIN2 (convergence criteria: energy change threshold $\leq$0.05 kcal/mol) and the standard TRIPOS force field.

The overlay model in Figure 2 was achieved using a MULTIFIT procedure, which performed a flexible fit between two molecules. Compound **8a** was built from the structure **7a** by replacing the methylene linkage with hydrogen. A default spring force constant (k) of 20 kcal/mol·Å^2 was applied in the multifit energy term ($E = \sum kd^2$). The resulting conformers were followed by unconstrained energy minimization and the rigid body least-squares FIT procedure.

Bioassay procedures

In vitro experiments. The binding assays for gastrin,[8] CCK-B, and CCK-A receptors[9] were as follows. 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.[10]

In vivo experiments. The determination of gastric acid secretion in anesthetized rats was achieved as follows. Gastric acid secretion was measured in anesthetized rats using Schild's rat method.[12] Acid secretion was measured at pH 7.0 by the addition of 0.01 N NaOH. When basal acid secretion had stabilized, histamine-2-HCl (3 mg kg^{-1} h^{-1}) was infused as acid via the vein. The test compounds were intraduodenally injected 90 min after the start of histamine infusion. Data are expressed as maximal inhibition.

Gastric acid inhibitory activity. Antisecretory activities were studied using pylorus ligation preparations.[13] 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 the automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0 (Hiranuma Comtite-7, Tokyo, Japan). Titratable acid output was expressed as μEq/2 h. The test compound or vehicle alone was given by po at 10 mg kg^{-1} at 1 h before ligating the pylorus.

The experimental procedure of the canine Heidenhain pouch method was based on ref 14.

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