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Synthesis and Structure–Activity Relationships of Dual Histamine H_2 and Gastrin Receptor Antagonists with Decreased Hydrophobicity

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Abstract—In order to study structure–activity relationships of the previously reported dual histamine H_2 and gastrin receptor antagonists and also to improve their low oral absorbability, we tried two chemical modifications. One tried to decrease the high hydrophobicity of the parent hybrid compounds to an appropriate level by incorporating a hydrophilic group into the molecule and the other by replacing the more hydrophobic groups with less hydrophobic ones. The former compounds (type I) involved hybrid compounds with a hydroxyl group at a position of a spacer, a piperidine moiety of H_2A, or a phenyl ring at the C_5 of the benzodiazepine skeleton as well as those with a free carboxyl group in the piperidine moiety of H_2A. The latter (type II) involved hybrid compounds with the C_5-phenyl group replaced with either a methyl group or hydrogen atom. Among them, only a type I compound, ({2-[3-(3-piperidin-1-ylmethylphenoxy)propylcarbamoyl]ethylcarbamoyl}methyl)carbamic acid 3-{3-[5-(3-hydroxyphenyl)-1-methyl-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]ureido}benzyl ester (**18**), showed potent dual histamine H_2 and gastrin receptor antagonistic activity, whereas others resulted in a significant decrease of histamine H_2 receptor antagonistic activity. The in vivo gastric acid antisecretory activity of **18** evaluated by Schild's rat method, however, did not suggest any notable improvement in oral absorbability. © 1997 Elsevier Science Ltd.

Introduction

In previous papers[1–3] we reported on the dual histamine H_2 and gastrin receptor antagonists (dual H_2 and G-A) and the design, synthesis, and pharmacological evaluation of joint-type hybrid molecules composed of two pharmacophore moieties taken from known histamine H_2 and gastrin receptor antagonists (H_2A and GA). Some compounds showed weak but distinct dual H_2 and G-A activity, revealing the importance of spacers and binding sites of both pharmacophores for the dual activity.[1] Subsequently, we found that modification of spacers and alteration of their binding modes at the GA site from the amide bond to the carbamate bond significantly improved not only their dual activity but also the GA versus CCK-A receptor selectivity.[2] Compound **1**[2] was one of the most desirable compounds in this class, satisfying all our in vitro activity standards. However, the oral in vivo gastric acid antisecretory activity of **1** was found to be much lower than its iv counterpart, indicating its low oral absorbability. In order to study structure–activity relationships of these dual antagonists in more detail and also to overcome the obstacle of their low oral absorbability, we synthesized a different type of dual antagonists which were constructed from H_2A and GA pharmacophore moieties by connecting them in a reversed head-to-head manner unlike the previously used head-to-tail manner. Among them, compound **2** with a substantially lower molecular weight than the previous compound **1** suggested some improvement in oral absorbability. However, that improvement was quite insufficient and furthermore its in vivo gastric acid antisecretory activity was very low.[3] Thus, we decided to try two chemical approaches from a different view point to study structure–activity relationships of dual H_2 and G-A. One was to decrease high hydrophobicity of the dual antagonists to a balanced level by incorporating a hydrophilic group into the molecule and the other to

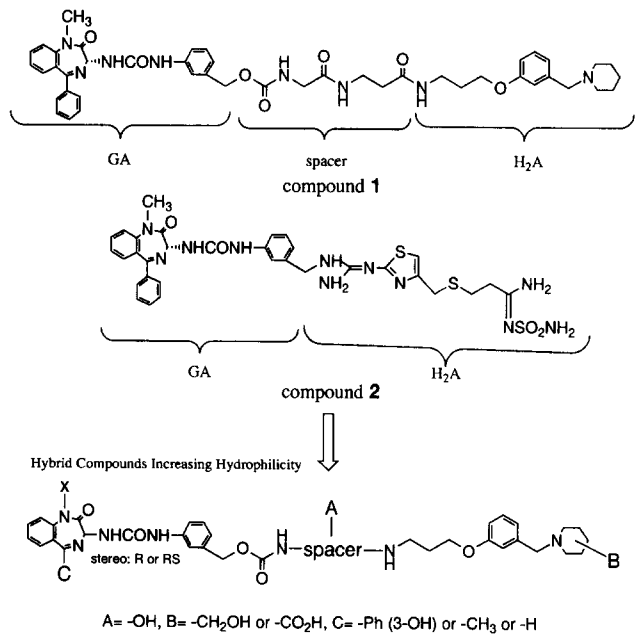


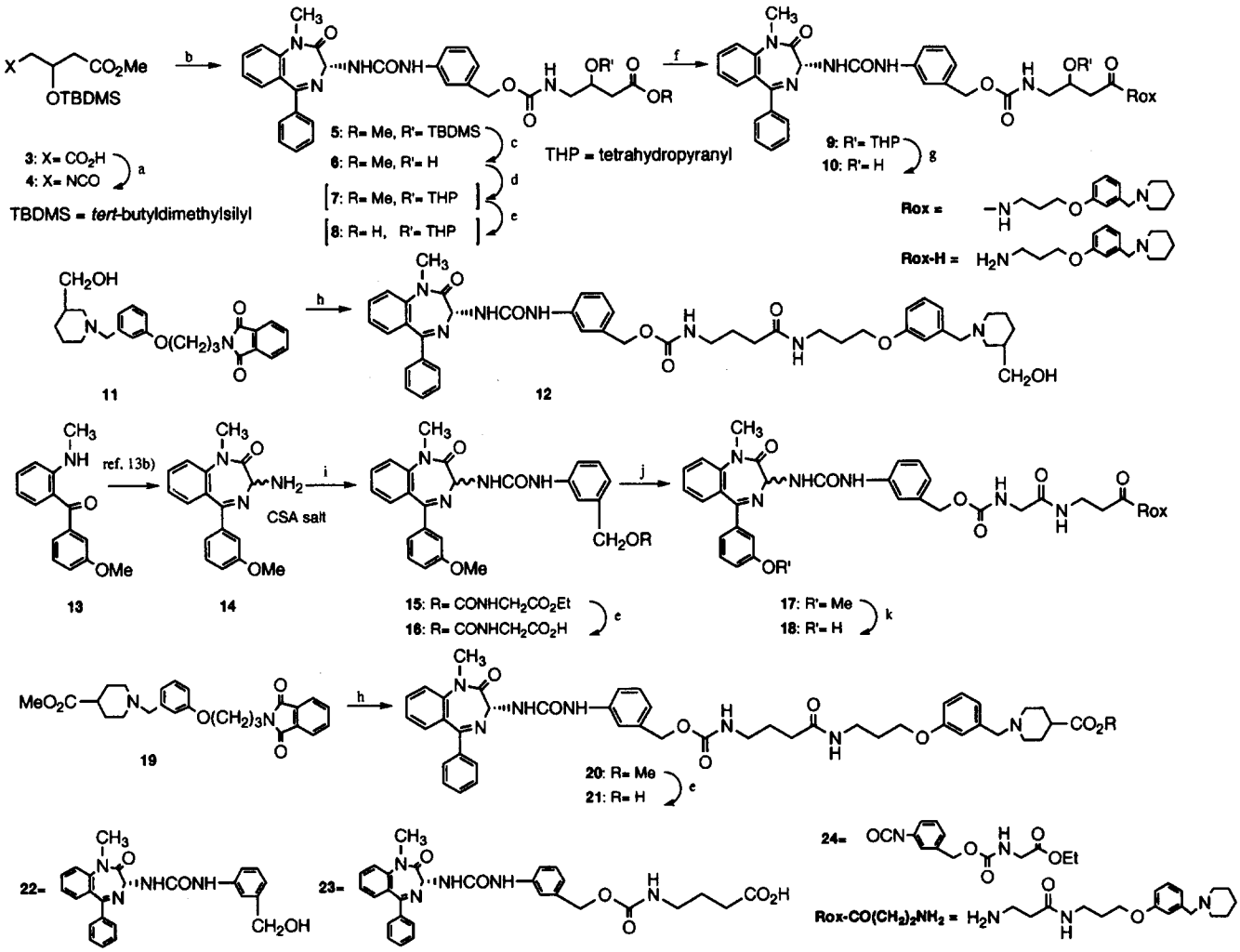
Figure 1.

decrease their hydrophobicity by replacing more hydrophobic groups involved in the molecule with less hydrophobic ones. In this article we describe the synthesis and evaluation of hybrid molecules incorporated with a hydroxyl or carboxyl group into the spacer part, the piperidine ring of the roxatidine moiety, or the phenyl ring at the benzodiazepine C_5 position. We also report on the substitution of the phenyl group at the C_5 position with a hydrogen atom or methyl group.

Chemistry

We synthesized two types of hybrid compounds. Type I involves not only hybrid compounds with a hydroxyl group incorporated into a spacer part, the piperidine ring of H_2A, or the phenyl ring at the C_5 of benzodiazepine skeleton but also those with a free carboxyl group incorporated into the piperidine ring of H_2A. Type II has the C_5-phenyl group replaced with either a methyl group or hydrogen atom. The molecular structures and synthetic schemes of type I and II compounds are summarized in Schemes 1 and 2, respectively.

Hybrid compound **10** bearing a hydroxyl group at the middle part of the propylene spacer was prepared from the starting material, methyl 3-(*tert*-butyldimethylsilyloxy)glutarate **3**[4] as follows (see Scheme 1). Curtius reaction of compound **3** gave isocyanate derivative **4** as usual, and the resultant **4** was converted to carbamate derivative **5** by treatment with benzodiazepine alcohol derivative **22** in the presence of bis(tri-*n*-butyltin)oxide ($(n\text{-}Bu_3Sn)_2O$) as a catalyst. After its *tert*-butyldimethylsilyl hydroxyl protecting group was exchanged with the tetrahydropyranyl group (compounds **5**, **6**, and **7**), ester **7** was hydrolyzed, without isolation, under alkaline conditions to give the free carboxylic acid derivative **8**. The reason for this replacement of the hydroxyl protective group at this stage was because the use of



a: 1) $ClCO_2{}^iBu$, Et_3N 2) NaN_3 3) heat b: $(n\text{-}Bu_3Sn)_2O$, **22** c: $n\text{-}Bu_4NF$ d: 2,3-dihydropyran, p-toluenesulfonic acid monohydrate e: aq. NaOH f: **Rox-H**, HOBt, Et_3N, WSCI g: aq.AcOH h: 1) hydrazine monohydrate 2) **23**, HOBt, Et_3N, WSCI i: **24**, Et_3N j: **Rox-CO(CH$_2$)$_2$NH$_2$**, HOBt, Et_3N, WSCI k: BBr_3

Scheme 1.

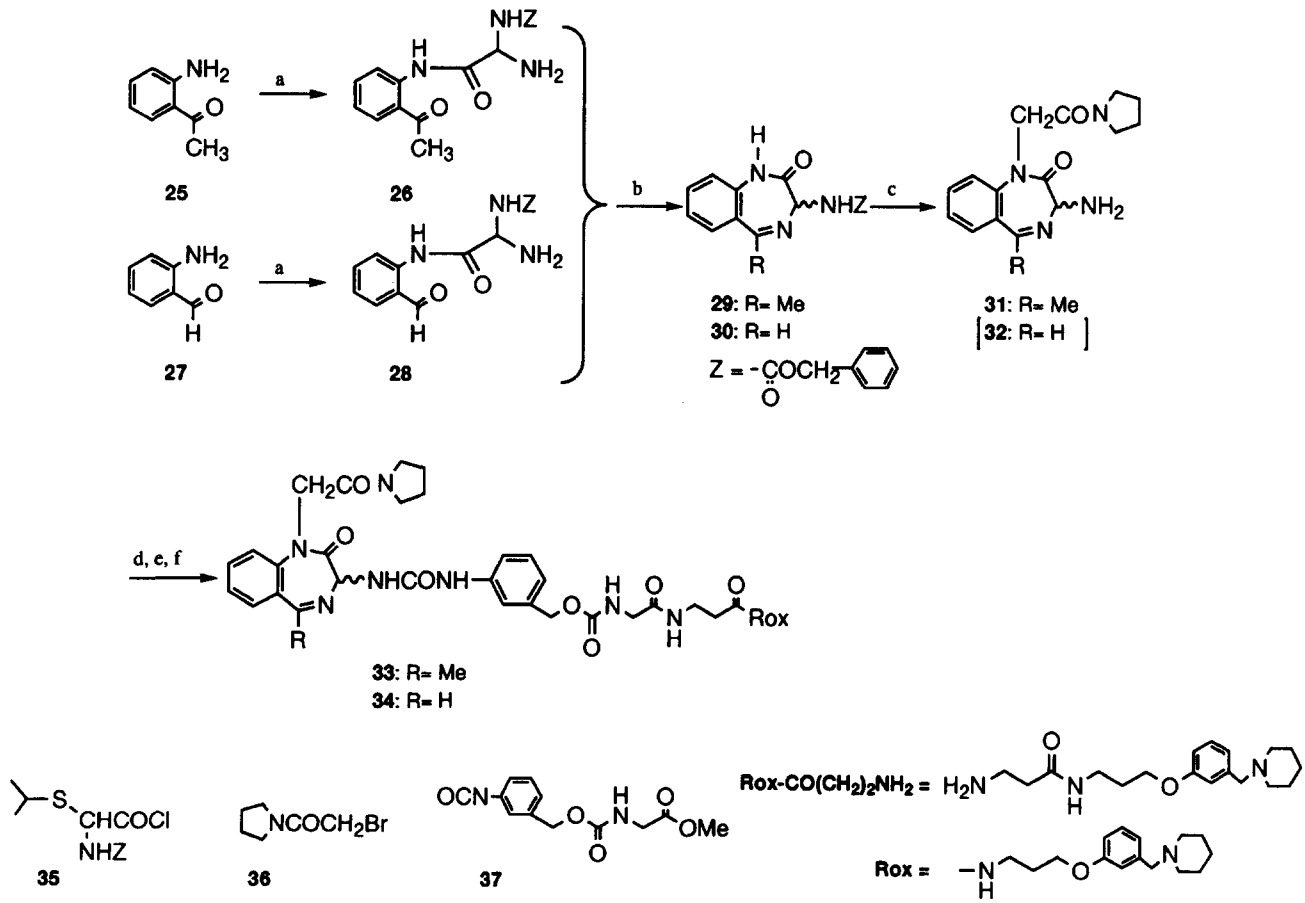
the *tert*-butyldimethylsilyl group resulted in cleavage of the carbamate group under these conditions to recover the starting alcohol derivative **22**. The carboxylic acid **8** was coupled without isolation with an amine derivative of roxatidine Rox-H to afford compound **9**. Finally, treatment of **9** with aqueous acetic acid afforded the desired hybrid compound **10** with a free hydroxyl group in the spacer position.

Hybrid compound **12** bearing the hydroxymethyl function at the piperidine C_3 position was prepared from the previously reported piperidine derivative **11**[3] by deprotecting its amino-protecting phthalimide group with hydrazine monohydrate followed by coupling the resultant free amine derivative with benzodiazepine carboxylic acid derivative **23** using water-soluble carbodiimide as a coupling agent.

To prepare hybrid compound **18** with a hydroxyl group incorporated into the meta position of the phenyl group at the benzodiazepine C_5 position, the starting material 3′-methoxy-2-methylaminobenzophenone **13** was prepared from 3-methoxybenzaldehyde and *N*-methylaniline by Sugasawa reaction[5] using borontrichloride (BCl_3) as a catalyst. It was then converted to racemic 3-amino-5-phenylbenzodiazepine camphorsulfonic acid salt **14** in almost the same way as previously reported.[3] Racemic **14** was coupled with isocyanate **24** to give an ester compound **15**. It was hydrolyzed to the acid **16** under alkaline conditions, followed by coupling with roxatidine amine derivative Rox-$CO(CH_2)_2NH_2$ as described above to produce hybrid compound **17**. Its ether cleavage with borontribromide (BBr_3) led to the hybrid compound **18**.

Hybrid compound **21** bearing the carboxylic acid group at the C_4-position of the piperidine ring was prepared from the piperidine derivative **19** which was prepared in almost the same way as reported for the *tert*-butyl ester in the previous paper[3] by a sequence of reactions: deprotection of its amino-protecting phthalimide group by hydrazine monohydrate, coupling the resultant free amine intermediate with benzodiazepine carboxylic acid derivative **23**, and ester hydrolysis under alkaline hydrolysis to give hybrid compound **21**.

Type II compounds **33** and **34** were designed to examine the pharmacological effects of replacing the phenyl group at the benzodiazepine C_5 position with a methyl group and a hydrogen atom, respectively. To prevent complete loss of the GA activity in these modifications, the methyl group at the N_1 position was replaced with a



Scheme 2.

slightly more hydrophobic group, pyrrolidinocarbonylmethyl group, which had been introduced by the Merck group[6a] to maintain their GA activities in the design of benzolactam-type gastrin antagonists.[6b] In addition, the same synthetic procedure as that used for the derivatives with the benzodiazepine C_5-phenyl group could not be successfully applied to these cases. Thus, an alternative procedure reported by the Merck group had to be employed to prepare compounds **33** and **34** from their corresponding starting materials, 2-aminoacetophenone **25** and 2-aminobenzaldehyde **27** as shown in Scheme 2. We describe here only the synthetic details of **33**. First, 2-aminoacetophenone **25** was treated with acid chloride **35**, followed by treatment with gaseous ammonia (NH_3) in the presence of mercury (II) chloride ($HgCl_2$) to give the α-aminoglycineamido intermediate **26**. Cyclization of this intermediate in glacial acetic acid containing ammonium acetate afforded a cyclized 5-methyl benzodiazepine derivative **29** in good yield. It was converted to **31** by treatment with the bromide **36** in the presence of potassium hydroxide (KOH) and tetrabutylammonium bromide (TBAB), followed by reductive removal of the benzyloxycarbonyl protecting group over 10% palladium-on-charcoal catalyst. Unfortunately, however, this reductive deprotection procedure could not be applied to the hydrogen-substituted compound **30** which gave very complex product mixtures. After some effort, we achieved efficient removal of the benzyloxycarbonyl by treatment with hydrogen fluoride (HF) in the presence of anisole to obtain compound **32** in good yield. Both compound **31** and **32** were then converted to the desired hybrid compounds **33** and **34** by the procedure used for the conversion of compound **14** to **17**, respectively.

Biological Results and Discussion

The assay[7-9] results, calculated hydrophobic parameters *Clog*P values, and chemical structures of type I and type II hybrid compounds are summarized in Tables 1 and 2, respectively. The *Clog*P values were easily calculated using an appropriate computer program.[10]

The results in Table 1 show that the *Clog*P values of type I compounds such as the hydroxylated compounds **10**, **12**, and **18**, and the carboxylated compound **21** are significantly lower than those of the parent compounds **A** and **B** (compound **1**), indicating that the introduction of either the hydroxyl or carboxyl group into the spacer part, piperidine ring, or phenyl group at the C_5 position substantially decreases their hydrophobicity. Given this theoretical information, we discuss here the structure–activity relationship of the type I compounds. First, introduction of the hydroxyl group into the spacer part or the piperidine ring resulted in great loss of H_2A activity as disclosed by the low pA_2 values of compounds **10** and **12**, <5.0 and 5.7, respectively. However, introduction of the hydroxyl group into the meta position of the phenyl group at C_5 did not decrease the pA_2 value of compound **18**, as clearly shown by its value of 6.6. Most surprising to us was the effect of introducing the carboxylic group into the hybrid compounds. This resulted in the total loss of the binding affinity of hybrid compounds with the histamine H_2 receptor as shown by the extremely low pA_2 value (<5.0) of compound **21**.

The GA activities of these hybrid compounds were well retained as shown by their potent IC_{50} values in Table 1 ranging between 18 nM for **12** and 38 nM for **10**.

We do not know why introduction of the hydroxyl or carboxyl group into these hybrid molecules affected mainly their pA_2 but not GA values. One possible reason is that spacer parts may play a crucial role as a hydrophobic pharmacophore moiety requisite for H_2A activity as has been suggested in the literature.[11] Also, the introduction of the carboxylic acid into the head amine pharmacophore may have decreased the binding affinity with the histamine H_2 receptor and/or retarded the formation of the active conformation of the molecule by intramolecular formation of a zwitter ion complex.

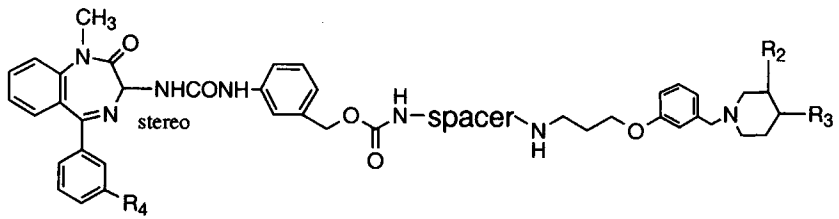
The results in Table 2 clearly show that the *Clog*P values of compounds **33** and **34** are significantly lower than those of the corresponding prototype compound **C**. Particularly, substitution of the phenyl group with hydrogen sharply decreases the *Clog*P value of the hybrid compound from 6.112 to 2.401. These data strongly indicate that substitution of the phenyl group at the C_5 position with the less hydrophobic methyl group or hydrogen atom would diminish the hydrophobicity of the parent hybrid compound to a certain extent. Hybrid compounds **33** and **34** showed a somewhat diminished but distinct pA_2 value of 5.9. However, these compounds showed very low GA activity (IC_{50} values of 110 and 620 nM, respectively), despite the use of the pyrrolidinocarbonylmethyl group instead of the methyl group as an N_1 substituent group. Thus, our attempts to obtain potent compounds with decreased hydrophobicity in this class were not fruitful.

After all, we focused on compound **18**, as it showed potent H_2A and GA activity with a pA_2 value of 6.6 and an IC_{50} of 23 nM, respectively. Furthermore, its GA versus CCK-A receptor selectivity was well retained. We expected that it would show some improvement in oral absorbability as a result of its somewhat diminished hydrophobicity. However, contrary to expectation, its in vivo gastric acid antisecretory activity evaluated by Schild's rat method[12] at 10 mg/kg dose for the id route was very low, only 20% inhibition, indicating very poor oral absorbability of this compound.

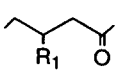
Conclusion

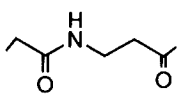
In this study, we tried two chemical modifications to study structure–activity relationships of the dual H_2 and G-A and also to improve their oral absorbability. One was to decrease very high hydrophobicity of the hybrid compounds to an appropriate level by incorporating a

Table 1. In vitro biological activities of hybrid compounds



								Receptors IC_{50} (nM)			Ratio	pA_2
No.	**R_1**	**R_2**	**R_3**	**R_4**	**Stereo**	**Spacer**	***Clog P***	**Gastrin**	**CCK-B**	**CCK-A**	**Gastrin:CCK-A**	**Histamine H_2**
A	H	H	H	H	R	I	5.785	26	94	3600	138	6.6
10	OH	H	H	H	R	I	5.549	38	82	520	14	<5.0
12	H	CH_2OH	H	H	R	I	4.317	18	42	460	26	5.7
21	H	H	CO_2H	H	R	I	4.228	24	100	1700	71	<5.0
1(=B)	—	H	H	H	R	II	5.740	19	103	8200	432	6.8
18	—	H	H	OH	RS	II	5.073	23	380	2700	117	6.6
	Cimetidine											6.6
	L-365.260							4	29	11100	2775	

Spacer I: 

spacer II: 

hydrophilic group into the molecule and the other by replacing the more hydrophobic phenyl group in the molecule with less hydrophobic ones (hydrogen and the methyl group). Among them, we found that compound **18** with a substantially decreased hydrophobic character than the parent compound **1** showed potent dual H_2 and G-A activity in vitro. However, any substantial improvement of oral absorbability was not observed with this compound.

We are continuing other studies on structure–activity relationships of dual H_2 and G-A in order 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. The ^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

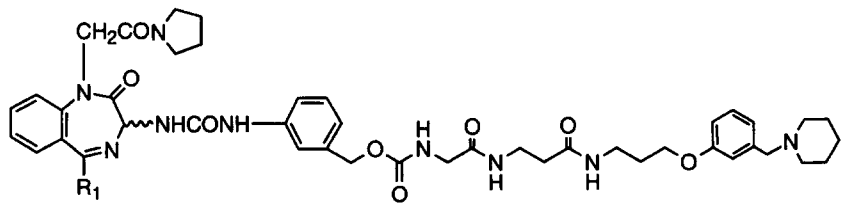
(*R*)-{2-Hydroxy-3-[3-(3-piperidin-1-ylmethylphenoxy)-propylcarbamoyl]propyl}carbamic acid 3-[3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)-ureido]benzyl ester (10).

Compound 7. To a solution of **6** (120 mg, 0.21 mmol) and dihydropyran (21 mg, 0.25 mmol) in 10 ml of methylene chloride (CH_2Cl_2) was added *p*-toluenesulfonic acid (TsOH) monohydrate (0.4 mg, 0.0021 mmol) at 20 °C, and the mixture was maintained for 2 h. The solution was diluted with 10 ml of CH_2Cl_2 and washed with saturated sodium bicarbonate ($NaHCO_3$) and with H_2O. The organic phase was dried over magnesium sulfate ($MgSO_4$) and concentrated in vacuo. Residue **7** was used as a starting material in the next step without purification.

Compound 8. Into an ice-cooled solution of **7** (80 mg, 0.12 mmol) in 1 ml of methanol (MeOH) was added an aqueous solution of sodium hydroxide (NaOH) (9.6 mg, 0.24 mmol) in 1 ml of H_2O with stirring. After stirring for 2 h at this temperature, the reaction mixture was neutralized with 1 N aqueous hydrochloric acid solution (1 N HCl) and evaporated in vacuo. The residue was extracted with ethyl acetate (EtOAc). The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated. The residue was used for the next step without purification.

Compound 9. 1-Hydroxybenzotriazol (HOBt) (12.6 mg, 0.093 mmol), triethylamine (Et_3N) (28 mg, 0.28 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSCI) (23 mg, 0.12 mmol) were added stepwise under ice cooling into a well stirred 2 ml of *N,N*-dimethylformamide (DMF) solution containing the amine derivative of roxatidine Rox-H (38.7 mg, 0.12 mmol) and benzodiazepine carboxylic acid derivative **8** (60 mg, 0.093 mmol). The reaction mixture was kept at room temperature and stirred for 16 h, and concentrated under vacuum. The residue was extracted with CH_2Cl_2 and washed with aq $NaHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated under vacuum. The residue was chromatographed on a silica gel column using chloroform ($CHCl_3$)–methanol (MeOH) (10:1, v/v) as an eluent to give **9** (62.5 mg, 77% from **8**) as a foam. ^1H NMR (CD_3OD) δ: 1.31–2.04 (m, 16H, $8CH_2$), 2.30–2.52 (m, 4H, $2CH_2$), 3.23–3.56 (m, xH, nCH_2+CH_3OH in CD_3OD), 3.48 (s, 3H, N-

Table 2. In vitro biological activities of hybrid compounds



No.	R_1	*ClogP*	Receptors IC_{50} (nM)			Ratio	pA_2
			Gastrin	CCK-B	CCK-A	Gastrin:CCK-A	Histamine H_2
33	$-CH_3$	5.552	110	820	58	<1	5.9
34	–H	2.401	620	>1000	<100	<1	5.9
	Cimetidine						6.6
	L-365,260		4	29	11100	2775	

Compound **C** (R_1 = C_6H_5): *ClogP* = 6.112.

CH_3), 3.80–4.62 (m, 4H, $2CH_2$), 5.48 (br, 1H, 3-H), 6.78–7.69 (m, 17H, Ar-H).

Compound 10. A mixture of **9** (60 mg, 0.069 mmol), acetic acid (0.9 ml), H_2O (0.3 ml) and tetrahydrofuran (THF) (0.3 ml) was stirred for 2 h at room temperature, and stirring was continued for 16 h at 0 °C. The reaction mixture was chromatographed on a silica gel column using $CHCl_3$–MeOH (10:1, v/v) as an eluent to give a foam substance **10** (22.5 mg, 41%). ^1H NMR (CD_3OD) δ: 1.35–2.00 (m, 10H, $5CH_2$), 2.30–2.50 (m, 4H, $2CH_2$), 3.15–3.50 (m, xH, nCH_2+CH_3OH in CD_3OD), 3.49 (s, 3H, N-CH_3), 3.80–4.80 (m, 4H, $2CH_2$), 5.35 (s, 1H, 3-H), 6.80–7.80 (m, 17H, Ar-H). Anal. calcd for $C_{44}H_{51}N_7O_7 \cdot H_2O$: C, 65.41; H, 6.61; N, 12.14. Found: C, 65.70; H, 6.83; N, 11.83.

(*R*)-(3-{3-[3-(3-Hydroxymethylpiperidin-1-ylmethyl)-phenoxy]propylcarbamoyl}propyl)carbamic acid 3-[3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)ureido]benzyl ester (12). The mixture solution of **11** (230 mg, 0.56 mmol) and hydrazine monohydrate (85 mg, 1.69 mmol) in ethanol (EtOH) (3 ml) was refluxed for 2 h and cooled. The reaction mixture was concentrated in vacuo and extracted with EtOAc. The organic layer was washed with 1 N NaOH and H_2O, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to the following coupling reaction, carried out by the method used for compound **9**, to give compound **12** as a foam substance. ^1H NMR (CD_3OD) δ: 1.50–2.10 (m, 11H, CH+$5CH_2$), 2.20 (t, 2H, CH_2, J = 8 Hz), 2.80–3.60 (m, xH, nCH_2+CH_3OH in CD_3OD), 3.49 (s, 3H, N-CH_3), 3.93 (t, 2H, CH_2, J = 7 Hz), 5.02 (s, 2H, CH_2), 5.35 (s, 1H, 3-H), 6.80–7.80 (m, 17H, Ar-H). Anal. calcd for $C_{45}H_{53}N_7O_7 \cdot 3H_2O$: C, 63.14; H, 6.93; N, 11.45. Found: C, 63.29; H, 6.62; N, 11.57.

({2-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-ethylcarbamoyl}methyl)carbamic acid 3-{3-[5-(3-hydroxyphenyl)-1-methyl-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]ureido}benzyl ester (18). To a solution of **17** (275 mg, 0.33 mmol) in 30 ml of CH_2Cl_2 was dropwise added at −60 °C a solution of boron tribromide (BBr_3) (1.22 g, 4.78 mmol) in 2 ml of CH_2Cl_2, with the reaction mixture kept at −30 °C for 3 h. Next, ice-cooled water was added, and the products were extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on a silica gel column using $CHCl_3$–MeOH (5:1, v/v) as an eluent to give **18** (109 mg, 40%) as a foam. Softening point 120–122 °C. ^1H NMR (CD_3OD) δ: 1.43–1.73 (m, 6H, $3CH_2$), 1.86–2.01 (m, 2H, CH_2), 2.31–2.43 (m, 2H, CH_2), 2.57–2.73 (m, 4H, $2CH_2$), 3.26–3.52 (m, 4H, $2CH_2$), 3.49 (s, 3H, N-CH_3), 3.59 (s, 2H, CH_2), 3.63–3.75 (m, 2H, CH_2), 3.91–4.03 (m, 2H, CH_2), 5.05 (s, 2H, CH_2), 5.34 (s, 1H, 3-H), 6.82–7.77 (m, 16H, Ar-H). Anal. calcd for $C_{45}H_{52}N_8O_8 \cdot 4.4H_2O$: C, 59.25; H, 6.72; N, 12.29. Found: C, 59.47; H, 6.44; N, 12.02.

(*R*)-1-{3-[3-(4-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)ureido]benzyloxycarbonylamino}butyrylamino)propoxy]benzyl}piperidine-4-carboxylic acid (21). Compound **21** was prepared by the method used for compound **8**. Softening point 162–164 °C. $[\alpha]^{25}_D$ −1.8 (*c* 1.005; MeOH). ^1H NMR (CD_3OD) δ: 1.69–2.23 (m, 10H, $5CH_2$), 2.25–2.63 (m, 1H, CH), 2.91–3.16 (m, 4H, $2CH_2$), 3.26–3.44 (m, 4H, $2CH_2$), 3.48 (s, 3H, N-CH_3), 4.00 (m, 2H, CH_2), 4.16 (s, 2H, CH_2), 5.01 (s, 2H, CH_2), 5.35 (s, 1H, 3-H), 6.92–7.76 (m, 17H, Ar-H). Anal calcd for $C_{45}H_{51}N_7O_8 \cdot 2.4H_2O$: C, 62.76; H, 6.53; N, 11.39. Found: C, 62.54; H, 6.35; N, 11.34.

({2-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-ethylcarbamoyl}methyl)carbamic acid 3-{3-[5-methyl-2-oxo-1-(2-oxo-2-pyrrolidin-1-ylethyl)-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]ureido}benzyl ester (33). To an ice-cooled solution of **31** (39 mg, 0.13 mmol) in 2 ml of CH_2Cl_2 was added Et_3N (0.055 ml, 0.39 mmol) and isocyanate **37** (37 mg, 0.14 mmol), and the reaction mixture was kept at the same temperature for 3 h. The reaction mixture was concentrated, and the residue was extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated. The residue, without purification, was subjected to hydrolysis of the ethyl ester and coupling reaction by the method used for compounds **8** and **9**. Softening point 130–132 °C. ^1H NMR ($CDCl_3$) δ: 1.39–2.04 (m, 10H, $5CH_2$), 2.09–2.43 (m, 4H, $2CH_2$), 2.45 (d, 3H, CH_3, J = 1.4 Hz), 2.52–2.73 (m, 4H, $2CH_2$), 3.23–3.54 (m, 8H, $4CH_3$), 3.67 (s, 2H, CH_2), 3.68–3.80 (m, 2H, CH_2), 3.91–4.03 (m, 2H, CH_2), 4.35, 4.62 (ABq, 2H, CH_2, J = 16.4 Hz, Δν = 53.4 Hz), 4.97 (s, 2H, CH_2), 5.41 (d, 1H, 3-H, J = 8 Hz), 6.74–7.59 (m, 12H, Ar-H). Anal. calcd for $C_{45}H_{57}N_9O_8S \cdot 4.6H_2O$: C, 57.81; H, 7.14; N, 13.49. Found: C, 57.90; H, 6.92; N, 13.21.

({2-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-ethylcarbamoyl}methyl)carbamic acid 3-{3-[2-oxo-1-(2-oxo-2-pyrrolidin-1-yl-ethyl)-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]ureido}benzyl ester (34). Compound **34** was prepared by the method used for compound **33**. Softening point 102–104 °C. ^1H NMR ($CDCl_3$) δ: 1.33–1.67 (m, 6H, $3CH_2$), 1.73–2.00 (m, 6H, $3CH_2$), 2.25–2.48 (m, 6H, $3CH_2$), 3.20–3.46 (m, 10H, $5CH_3$), 3.71 (t, 2H, CH_2, J = 8 Hz), 3.91 (t, 2H, CH_2, J = 8 Hz), 4.29, 4.68 (ABq, 2H, CH_2, J = 16.4 Hz, Δν = 77.7), 4.94 (s, 2H, CH_2), 5.44 (d, 1H, 3-H, J = 5.4 Hz), 6.62–6.87 (m, 5H, $2CH_2$+NH), 7.00–7.51 (m, 12H, Ar-H), 8.51 (s, 1H, 5-H). Anal. calcd for $C_{44}H_{55}N_9O_8$: C, 63.06; H, 6.62; N, 15.05. Found: C, 63.21; H, 6.58; N, 15.21.

Preparation of intermediates used for the synthesis of hybrid compounds

3-(*tert*-Butyldimethylsilanyloxy)pentanedioic acid monomethyl ester (3). Compound **3** was prepared by a described method.[4] ^1H NMR ($CDCl_3$) δ: 0.08 (d, 6H, $2CH_3$, J = 3 Hz), 0.85 (s, 9H, *tert*-Bu), 2.57–2.63 (m,

4H, $2CH_2$), 3.68 (s, 3H, CH_3), 4.55 (q, 1H, CH, J = 6 Hz).

3-(*tert*-Butyldimethylsilanyloxy)-4-isocyanatobutyric acid methyl ester (4). Compound **4** was prepared by a previously described method.[3] IR ($CHCl_3$) cm^{-1}: 2250 (NCO).

(*R*)-3-(*tert*-Butyldimethylsilanyloxy)-4-{3-[3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)-ureido]benzyloxycarbonylamino}butyric acid methyl ester (5). Compound **5** was prepared by a described method.[3] ^1H NMR ($CDCl_3$) δ: 0.05 (d, 6H, $2CH_3$, J = 6 Hz), 0.85 (s, 9H, *tert*-Bu), 2.48–2.52 (m, 2H, CH_2), 3.20–3.24 (m, 2H, CH_2), 3.49 (s, 3H, N-CH_3), 3.67 (s, 3H, CH_3), 4.19–4.30 (m, 1H, CH), 5.04–5.06 (m, 2H, CH_2), 5.55 (d, 1H, 3-H, J = 8 Hz), 6.95–7.65 (m, 13H, Ar-H).

(*R*)-3-Hydroxy-4-{3-[3-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)ureido]benzyloxycarbonylamino}butyric acid methyl ester (6). Into a solution of **5** (129 mg, 0.188 mmol) in 2 ml of THF was added 1.0 M tetrabutylammonium fluoride (Bu_4NF) solution in THF (0.282 ml, 0.973 mmol) at −30 °C. The reaction mixture was kept at room temperature for 3 h, and the precipitates were collected by filtration and washed well with diethylether (Et_2O). The solid was dissolved in CH_2Cl_2 and washed with H_2O, dried over $MgSO_4$ and concentrated under vacuum. The residue was crystallized from acetone–Et_2O to give **6** (92 mg, 85%). mp 149–152 °C. ^1H NMR ($CDCl_3$) δ: 2.40–2.44 (m, 2H, CH_2), 3.03–3.20 (m, 2H, CH_2), 3.46 (s, 3H, N-CH_3), 3.68 (s, 3H, CH_3), 4.02–4.20 (m, 1H, CH), 5.04 (s, 2H, CH_2), 5.58 (d, 1H, 3-H, J = 8 Hz), 6.88–7.86 (m, 13H, Ar-H).

(3-{3-[5-(3-Methoylphenyl)-1-methyl-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]ureido}benzyloxycarbonyl-amino)acetic acid ethyl ester (15). Compound **15** was prepared by the method used for compound **33**. Softening point 154–156 °C. ^1H NMR ($CDCl_3$) δ: 1.27 (t, 3H, CH_3, J = 7.2 Hz), 3.47 (s, 3H, N-CH_3), 3.81 (s, 3H, OCH_3), 3.96 (d, 2H, CH_2, J = 5.4 Hz), 4.21 (q, 2H, CH_2, J = 7.2 Hz), 5.06 (s, 2H, CH_2), 5.57 (d, 1H, 3-H, J = 8 Hz), 6.93–7.64 (m, 12H, Ar-H). Anal. calcd for $C_{30}H_{31}N_5O_7 \cdot 0.2H_2O$: C, 62.42; H, 5.48; N, 12.13. Found: C, 62.29; H, 5.53; N, 12.09.

(3-{3-[5-(3-Methoylphenyl)-1-methyl-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]ureido}benzyloxycarbonyl-amino)acetic acid (16). Compound **16** was prepared by the method used for compound **8**. Softening point 136–137 °C. ^1H NMR ($CDCl_3$) δ: 3.43 (s, 3H, N-CH_3), 3.77 (s, 3H, OCH_3), 3.84 (m, 2H, CH_2), 5.04 (s, 2H, CH_2), 5.55 (d, 1H, 3-H, J = 8 Hz), 6.95–7.63 (m, 12H, Ar-H). Anal. calcd for $C_{28}H_{27}N_5O_7 \cdot 0.8H_2O$: C, 60.06; H, 5.15; N, 12.51. Found: C, 60.10; H, 4.95; N, 12.33.

({2-[3-(3-Piperidin-1-ylmethylphenoxy)propylcarbamoyl]-ethylcarbamoyl}methyl)carbamic acid 3-{3-[5-(3-methoylphenyl)-1-methyl-2-oxo-2,3-dihydro-1H-1,4-benzo-diazepin-3-yl]ureido}benzyl ester (17). Compound **17** was prepared by the method used for compound **9**. Softening point 114–116 °C. ^1H NMR ($CDCl_3$) δ: 1.40–1.75 (m, 6H, $3CH_2$), 1.80–2.03 (m, 2H, CH_2), 2.31–2.71 (m, 6H, $3CH_2$), 3.27–3.79 (m, 6H, $3CH_2$), 3.41 (s, 3H, N-CH_3), 3.62 (s, 2H, CH_2), 3.91–4.04 (m, 2H, CH_2), 5.03 (s, 2H, CH_2), 5.52 (d, 1H, 3-H, J = 8 Hz), 6.72–7.63 (m, 16H, Ar-H). Anal. calcd for $C_{46}H_{54}N_8O_8 \cdot 1.6H_2O$: C, 63.08; H, 6.58; N, 12.80. Found: C, 63.19; H, 6.48; N, 12.74.

(−)-(*R*)-1-{3-[3-(4-{3-[3-(1-Methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)ureido]benzyloxy-carbonylamino}butyrylamino)propoxy]benzyl}piperidine-4-carboxylic acid methyl ester (20). Compound **20** was prepared by the method used for compound **12**. Softening point 95–97 °C. $[\alpha]^{25}_D$ −1.0 (*c* 1.018, MeOH). ^1H NMR ($CDCl_3$) δ: 1.63–2.38 (m, 11H, CH+$5CH_2$), 2.81–2.94 (m, 2H, CH_2), 3.12–3.25 (m, 2H, CH_2), 3.31–3.50 (m, 4H, $2CH_2$), 3.43 (s, 3H, N-CH_3), 3.45 (s, 2H, CH_2), 3.66 (s, 3H, CH_3), 4.95 (m, 2H, CH_2), 5.02 (s, 2H, CH_2), 5.53 (d, 1H, 3-H, J = 8.2 Hz), 6.83–7.64 (m, 17H, Ar-H). Anal. calcd for $C_{46}H_{53}N_7O_8 \cdot 4.2H_2O$: C, 60.87; H, 6.82; N, 10.80. Found: C, 60.97; H, 6.55; N, 10.59.

(5-Methyl-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)-carbamic acid benzyl ester (29). Compound **29** was prepared by a described method.[13] ^1H NMR ($CDCl_3$) δ: 2.50 (s, 3H, CH_3), 5.13–5.21 (m, 3H, CH_2+3-H), 7.05–7.13 (m, 1H, Ar-H), 7.24–7.40 (m, 6H, Ar-H), 7.42–7.66 (m, 2H, Ar-H).

(2-Oxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl)carbamic acid benzyl ester (30). Compound **30** was prepared by a described method.[13] ^1H NMR ($CDCl_3$) δ: 5.15 (s, 2H, CH_2), 5.24–5.29 (m, 1H, 3-H), 7.07–7.11 (m, 1H, Ar-H), 7.26–7.39 (m, 6H, Ar-H), 7.50–7.57 (m, 2H, Ar-H), 8.54–8.55 (m, 1H, 5-H).

3-Amino-5-methyl-1-(2-oxo-2-pyrrolidin-1-yl-ethyl)-1,3-dihydro-1,4-benzodiazepin-2-one (31). To an ice-cooled suspension of **29** (307 mg, 0.95 mmol) and powdered potassium hydroxide (KOH) (69 mg, 1.24 mmol) and tetrabutylammonium bromide (TBAB) (306 mg, 0.95 mmol) in 3 ml of THF was added bromide **36** (237 mg, 1.24 mmol), and the reaction mixture was kept at the same temperature for 3 h. The precipitates were filtered off, and the filtrate was concentrated in vacuo. The residue was extracted with $CHCl_3$ and the organic layer was washed with 1 N HCl, 5% sodium carbonate (Na_2CO_3) and H_2O, dried over $MgSO_4$ and concentrated under vacuum. The residue was used for the next step without purification. The residue was dissolved in 10 ml of MeOH, and hydrogen gas was introduced in the presence of 10% palladium-on-charcoal (Pd–C) catalyst at atmospheric pressure and at room temperature for 3 h. After filtration, the filtrate was concentrated in vacuo to give crude **31** (198 mg). ^1H NMR ($CDCl_3$) δ: 1.80–2.08 (m, 4H, $2CH_2$), 2.49 (s, 3H, CH_3), 3.38–3.65 (m, 4H, $2CH_2$), 4.27, 4.74 (ABq, 2H, J = 16.2 Hz, $\Delta\nu$ = 95 Hz), 4.41–4.43 (m, 1H, 3-H), 7.21–7.58 (m, 4H, Ar-H).

Bioassay procedures

In vitro experiments

The binding assays for the gastrin,[7] CCK-B, and CCK-A receptor[8] are 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.[9]

In vivo experiments

Determination of gastric acid secretion in anesthetized rats. 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.

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