Structure–Antigastrin Activity Relationships of New (*R*)-4-Benzamido-5-oxopentanoic Acid Derivatives

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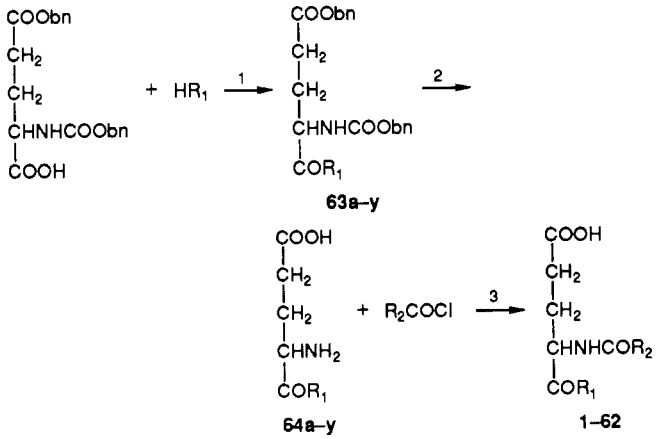
New (*R*)-4-benzamido-5-oxopentanoic acid derivatives were synthesized by a stereoconservative procedure and evaluated in vitro for their capacity to inhibit the binding of [^{125}I](BH)-CCK-8 to either rat peripheral (CCK-A) or central (CCK-B) CCK receptors, or the binding of [^{3}H]pentagastrin to rabbit gastric glands, as well as to inhibit, in vivo, the acid secretion induced by pentagastrin infusion in the perfused rat stomach. The parent compound of this series (lorglumide) is the first nonpeptidic, potent and selective antagonist of the CCK-A receptor. Chemical manipulations of the structure of lorglumide led to the discovery of selective antagonists of the CCK-B/gastrin receptors. Structure–activity relationships are discussed. Some of these new derivatives exhibit different affinities with rabbit gastric gland cells and rat cortex membranes, suggesting that the stomach gastrin receptor (arbitrarily termed CCK-B_1 receptor) is not as closely related to the CCK central receptor (termed CCK-B_2) as previously hypothesized. The antigastrin activity of the most potent compound of the series, i.e. (*R*)-4-(3,5-dichlorobenzamido)-5-(8-azaspiro-[4.5]decan-8-yl)-5-oxopentanoic acid (compound **28**, CR 2194) was further evaluated in vivo: in the first hour after administration the compound inhibits acid secretion induced by pentagastrin infusion, in both cat and dog (in the cat with gastric fistula and in the dog with Heidenhain pouch), with ID_{50}s (mg/kg) of 15.5 (iv) (cat), 8.7 (IV) (dog) and 24.2 (oral) (Heidenhain dog). The characteristics of CR 2194, that is, the selectivity for the gastrin receptor, the simple nonpeptidic molecular structure, and the activity after oral administration, indicate that this compound is a useful tool in the study of the biological effects of gastrin and a potential agent for diagnostic or therapeutic use.

The gastrointestinal polypeptide hormones gastrin and cholecystokinin (CCK) are closely related chemically, both having a common terminal pentapeptide amide sequence, but they exhibit different biological effects on their target tissues. For instance, in vivo, gastrin is a potent stimulant of acid gastric secretion and a very weak stimulant of pancreatic enzyme release,[1] while CCK is the main hormonal regulator of gallbladder contractility and of pancreatic enzyme secretion.[2] More recently it was demonstrated that CCK is also widely distributed in the brain and it was hypothesized that it may function as a neurotransmitter or neuromodulator in the central nervous system (CNS).[3,4] The peripheral actions of CCK are mediated by a receptor subtype termed CCK-A, while the central actions are mainly mediated by the subtype receptor termed CCK-B, for which the minimum agonist ligand requirement is tetragastrin (CCK-4).[5] A third receptor subtype, which appears to be closely related to the CCK-B type, is the stomach gastrin receptor.[6]

D,L-4-[(3,4-Dichlorobenzoyl)amino]-5-(dipentylamino)-5-oxopentanoic acid (lorglumide, CR 1409) is the first nonpeptidic, potent, competitive, and specific CCK-A antagonist, with low affinity with the CCK-B receptor, and is ineffective, even when administered in subtoxic doses, as an inhibitor of gastric secretion induced by pentagastrin in the rat.[7,8]

Scheme I.[a] Synthesis of Compounds 1–62 of Table I



[a] Reagents: (1) Et_3N, EtOCOCl; (2) H_2, 10% Pd on C; (3) NaOH. bn = benzyl.

Recently several potent and specific CCK-B antagonists have been discovered, for example, compounds L-365,260[9] or α-methyltryptophan derivatives (CI-988).[10]

The aim of our investigation was to explore the possibility that appropriate chemical manipulations of the structure of lorglumide could lead to new molecular entities

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0022-2623/92/1835-0028$03.00/0

exhibiting potent and selective antagonist activities on CCK-B and gastrin receptors.

Chemistry

Since it has been demonstrated that the CCK-antagonist activity of lorglumide is stereospecific and due essentially to the *R* form,[11] we have utilized in the synthesis of these new derivatives a method (illustrated by Scheme I) that allows retention of the configuration of glutamic acid. Therefore *N*-Cbz-γ-benzylglutamic acid (D or L) is condensed with the amine of formula R_1H to obtain intermediate **63(a–y)**. The Cbz and benzyl groups are removed together by reacting **63** dissolved in methanol with H_2 under room conditions in the presence of a catalytical amount of palladium on charcoal. The resultant amino acid **64(a–y)** is then converted to the final product (**1–62**) by acylation under Schotten–Baumann conditions with equivalent quantities of the appropriate acyl chloride of formula R_2COCl.

The physicochemical characteristics of the new 4-benzamido-5-oxopentanoic acid derivatives are given in Table I.

Results and Discussion

The results obtained from binding and gastrin antagonism are presented in Table II.

Initially we synthesized a homologous series of linear (C4–C7) alkyl secondary amides of *N*-benzoyl-D-glutamic acid, unsubstituted or with mono- and disubstituted chloro derivatives of the aromatic ring (compounds **1–7**). Among these derivatives the 3-chloro compound displayed the highest anti-gastrin activity in vivo in the perfused rat stomach (ED_{50} = 36 mg/kg), whereas, contrary to the SAR in the corresponding lorglumide CCK-antagonists series, the 3,4-dichloro substitution (compound **5**) does not increase the activity over that of the unsubstituted aromatic ring. The introduction in R_1 of an alkyl branched substituent at a distance of not less than three carbon atoms from the amidic nitrogen (compounds **8–19**) increases the antigastrinic activity in vivo.

Among these compounds the best substitution was obtained when R_1 was a (aminobutyl)-3,3-dimethyl group and R_2 was 3-chloro or 3,5-dichlorophenyl (compounds **10** and **12**), both showing an ED_{50} in vivo of 16 mg/kg.

It is worthy to note the complete loss of activity of compound **20**, carrying in R_1 a branched chain only two carbon atoms from the amidic nitrogen.

The introduction in R_1 of secondary cyclic (C6–C10 size) amides (compounds **21–25**) produces compounds with mild antigastrin activity. In order to exclude the hypothesis that the antigastrin activity of this series of 4-benzamido-5-oxopentanoic acid derivatives is linked to the presence in COR_1 of a secondary amido group, a COR_1 tertiary amide was synthesized in which R_1 was a 4,4-dimethylpiperidin-1-yl group and R_2 a 3-chlorophenyl group. This compound also exhibited a mild anti-gastrin activity in vivo (compound **26**, ED_{50} = 30 mg/kg).

Because of this encouraging result, a series of COR_1 tertiary amides was synthesized in which the amino group was an azaspirobicyclo group consisting of not more than 10 carbon atoms and having a maximum length of about 6 Å and a maximum width of about 3 Å (compounds **27–49**).

The best anti-gastrin activity in vivo in this series of derivatives is obtained when R_1 is the 8-azaspiro[4.5]decan-8-yl group (compounds **27–42**) and among such compounds the compounds in which R_2 is 3-chloro or 3,5-dichlorophenyl, showing an ED_{50} of 15 and 11 mg/kg, respectively (compounds **27** and **28**). The introduction of other halogen substituents in R_2 such as 2,3-dichloro, 3,4-dichloro, 3-Br, or 3-CF_3 groups is less favorable. The same happens with the introduction in R_2 of alkyl or alkoxy groups such as 3-Me, 3,5-Me_2, 3-Et, 4-iPr, and 3-OCH_3 (compounds **31–35**) as well as with the introduction of bulky groups such as 2-naphthyl, 3-quinolinyl, and 2-indolyl (compounds **40–42**) or especially with the introduction of electron-withdrawing groups such as 3-NO_2 and 3-CN (compounds **36** and **37**). Less effective are also the compounds in which R_1 is the group 2-azaspiro[4.4]nonan-2-yl (compound **47**), 2-azaspiro[4.5]decan-2-yl (compounds **48** and **49**), or 3-azaspiro[5.5]undecan-3-yl (compounds **43–46**).

Good anti-gastrin activity in vivo is obtained when R_1 is also a decahydroisoquinolin-2-yl group (compound **50**) or a 1-(aminoethyl)-2-(1′-adamantyl) group (compounds **51** and **52**) and in which R_2 is a 3-chloro- or 3,5-dichlorophenyl group. However, the secondary COR_1 amides in which R_1 is 3-aminospiro[5.5]undecane or 2-aminodecahydronaphthalene (compounds **53–55**) are all ineffective, probably because the R_1 group is, in this case, too bulky or because the spatial arrangement of the R_1 moiety is not suitable for interaction with the hydrophobic receptor surface.

As in the series of lorglumide CCK-A antagonists, with these new gastrin antagonists the activity is also stereospecific and attributable essentially to the *R* configuration. In fact the *S* enantiomers (compounds **56–60**) are all poorly effective or ineffective in comparison with the corresponding *R* enantiomers (compounds **10**, **27–29**, and **51**).

The situation is different with regard to the affinity with the peripheral CCK-A receptor (binding to the rat pancreatic acini). Generally, the COR_1 tertiary amides are much more effective than the corresponding secondary amides and higher activity is achieved when R_1 is a 3-azaspiro[5.5]decan-3-yl group and R_2 is a 3,4-dichlorophenyl, 2-naphthyl, or 3-quinolinyl group (compounds **44–46**).

These compounds are in fact very effective, displaying an IC_{50} of 0.4–0.6 μM, that is about 10 times higher than that exhibited by the two di-*n*-pentyl COR_1 amides (*R*)-lorglumide and CR 1795[12] (compounds **61** and **62**).

The most potent in vivo CCK-B antagonists of the series, for example compounds **10**, **12**, **27**, **28**, and **52**, are, on the other hand, very weak CCK-A antagonists, all being about 200–2000 times less potent than the specific CCK-A antagonist (*R*)-lorglumide.

With regard to the affinity with the central CCK-B receptor (binding to rat brain cortex) the situation is, once again, different.

Some of the most potent in vivo pentagastrin antagonists, for example compounds **28** and **52**, show a high affinity (IC_{50} = 0.6 and 0.3 μM, respectively) with the CCK-B receptor, but not higher than that exhibited by compound **45**, which is inactive in vivo as an anti-gastrin, or by the most potent CCK-A antagonist of the lorglumide series, i.e. compound **62**, which is completely ineffective on the same model in vivo. Furthermore, compounds **10** and **12**,

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Table I. Physical Properties of (*R*)-4-Benzamido-5-oxopentanoic Acid Derivatives (1–62) Prepared by Scheme I

$$HOOCCH_2CH_2\underset{\displaystyle NHCOR_2}{\overset{\displaystyle |}{C}}HCOR_1$$

compd	R_1	R_2	mp, °C	recryst solvent	optical[a] rotation, deg	% overall yield	formula	anal.
1	butylamino	3-ClC_6H_4	154	*i*-PrOH/H_2O 1:2	+22.8[b]	48	$C_{16}H_{21}ClN_2O_4$	C, H, N
2	pentylamino	C_6H_5	128	*i*-PrOH/H_2O 1:2	+33.2[b]	55	$C_{17}H_{24}N_2O_4$	C, H, N
3	pentylamino	3-ClC_6H_4	112	*i*-PrOH/H_2O 1:2	+29.1[b]	52	$C_{17}H_{23}ClN_2O_4$	C, H, N
4	pentylamino	4-ClC_6H_4	123	*i*-PrOH/H_2O 1:2	+22.8[b]	50	$C_{17}H_{23}ClN_2O_4$	C, H, N
5	pentylamino	3,4-$Cl_2C_6H_3$	162	MeCN	+17.2[b]	55	$C_{17}H_{22}Cl_2N_2O_4$	C, H, N
6	hexylamino	3-ClC_6H_4	128	*i*-PrOH/H_2O 1:2	+27.8[b]	44	$C_{18}H_{25}ClN_2O_4$	C, H, N
7	heptylamino	3-ClC_6H_4	135	*i*-PrOH/H_2O 1:2	+29.6[b]	43	$C_{19}H_{27}ClN_2O_4$	C, H, N
8	(3-methylbutyl)amino	3-ClC_6H_4	102	EtOH/H_2O 1:2	+24.0[b]	47	$C_{17}H_{23}ClN_2O_4$	C, H, N
9	(3,3-dimethylbutyl)amino	C_6H_5	132	*i*-PrOH/H_2O 1:3	+37.2[b]	57	$C_{18}H_{26}N_2O_4$	C, H, N
10	(3,3-dimethylbutyl)amino	3-ClC_6H_4	132	EtOH/H_2O 1:1	+21.7[b]	51	$C_{18}H_{25}ClN_2O_4$	C, H, N
11	(3,3-dimethylbutyl)amino	2,3-$Cl_2C_6H_3$	139	EtOH/H_2O 1:1	+19.3[b]	48	$C_{18}H_{24}Cl_2N_2O_4$	C, H, N
12	(3,3-dimethylbutyl)amino	3,5-$Cl_2C_6H_3$	140	EtOH/H_2O 1:1	+24.0[b]	53	$C_{18}H_{24}Cl_2N_2O_4$	C, H, N
13	(3,3-dimethylbutyl)amino	3,4-$Cl_2C_6H_3$	154	EtOH/H_2O 1:2	+14.0[b]	54	$C_{18}H_{24}Cl_2N_2O_4$	C, H, N
14	(3,3-dimethylbutyl)amino	2-naphthyl	160	EtOH/H_2O 1:1	+18.4[b]	58	$C_{22}H_{28}N_2O_4$	C, H, N
15	(3,3-dimethylbutyl)amino	2-indolyl	202	EtOH/H_2O 1:1	−8.4	45	$C_{20}H_{27}N_3O_4$	C, H, N
16[c]	(4,4-dimethylpentyl)amino	3-ClC_6H_4	155	EtOH/H_2O 1:1	+14.7[b]	37	$(C_{19}H_{26}ClN_2O_4)_2Ca$	C, H, N[d]
17	(4,4-dimethylpentyl)amino	3-quinolinyl	184	EtOH/H_2O 1:1	−14.0	40	$C_{22}H_{29}N_3O_4$	C, H, N
18	(3-ethyl-3-methylpentyl)amino	3-ClC_6H_4	131	EtOH/H_2O 1:2	+16.6[b]	40	$C_{20}H_{29}ClN_2O_4$	C, H, N
19	(3,3-diethylpentyl)amino	3-ClC_6H_4	115	EtOH/H_2O 1:1	+13.3[b]	38	$C_{21}H_{31}ClN_2O_4$	C, H, N
20	(2-ethylhexyl)amino	3-ClC_6H_4	85	EtOH/H_2O 1:1	+15.7[b]	43	$C_{20}H_{29}ClN_2O_4$	C, H, N
21	cycloheptylamino	3-ClC_6H_4	145	EtOH/H_2O 1:1	−2.4	52	$C_{19}H_{25}ClN_2O_4$	C, H, N
22	cyclooctylamino	3-ClC_6H_4	135	MeCN	+40.0[b]	47	$C_{20}H_{27}ClN_2O_4$	C, H, N
23	cyclodecylamino	3-ClC_6H_4	124	MeCN	−4.8	32	$C_{22}H_{31}ClN_2O_4$	C, H, N
24	(4,4-dimethylcyclohexyl)amino	3-ClC_6H_4	133	MeCN	+23.6[b]	44	$C_{20}H_{27}ClN_2O_4$	C, H, N
25	(4,4-diethylcyclohexyl)amino	3-ClC_6H_4	154	MeCN	+17.5[b]	39	$C_{22}H_{31}ClN_2O_4$	C, H, N
26	4,4-dimethylpiperidin-1-yl	3-ClC_6H_4	85	MeCN	−20.8[b]	50	$C_{19}H_{25}ClN_2O_4$	C, H, N
27	8-azaspiro[4.5]decan-8-yl	3-ClC_6H_4	150	MeCN	−17.0	60	$C_{21}H_{27}ClN_2O_4$	C, H, N
28	8-azaspiro[4.5]decan-8-yl	3,5-$Cl_2C_6H_3$	186	MeCN/MeOH 6:1	−17.4	61	$C_{21}H_{26}Cl_2N_2O_4$	C, H, N
29	8-azaspiro[4.5]decan-8-yl	3,4-$Cl_2C_6H_3$	151	MeCN	−22.0	68	$C_{21}H_{26}Cl_2N_2O_4$	C, H, N
30	8-azaspiro[4.5]decan-8-yl	2,3-$Cl_2C_6H_3$	167	MeCN	−26.1[b]	63	$C_{21}H_{26}Cl_2N_2O_4$	C, H, N
31	8-azaspiro[4.5]decan-8-yl	3-MeC_6H_4	150	MeCN	−17.1	53	$C_{22}H_{30}N_2O_4$	C, H, N
32	8-azaspiro[4.5]decan-8-yl	3-EtC_6H_4	120	MeCN	−14.9	48	$C_{23}H_{32}N_2O_4$	C, H, N
33	8-azaspiro[4.5]decan-8-yl	3,5-$Me_2C_6H_3$	185	EtOH/H_2O 1:1	−17.2	47	$C_{23}H_{32}N_2O_4$	C, H, N
34	8-azaspiro[4.5]decan-8-yl	4-*i*-PrC_6H_4	158	MeCN	−23.1	48	$C_{24}H_{34}N_2O_4$	C, H, N
35	8-azaspiro[4.5]decan-8-yl	3-$OMeC_6H_4$	116	MeCN	−15.8	36	$C_{22}H_{30}N_2O_5$	C, H, N
36	8-azaspiro[4.5]decan-8-yl	3-$NO_2C_6H_4$	183	MeCN	−16.6	53	$C_{21}H_{27}N_3O_6$	C, H, N
37	8-azaspiro[4.5]decan-8-yl	3-CNC_6H_4	155	MeCN	−20.1	45	$C_{22}H_{27}N_3O_4$	C, H, N
38	8-azaspiro[4.5]decan-8-yl	3-BrC_6H_4	146	MeCN	−14.9	45	$C_{21}H_{27}BrN_2O_4$	C, H, N
39	8-azaspiro[4.5]decan-8-yl	3-$CF_3C_6H_4$	123	MeCN	−14.5	39	$C_{22}H_{27}F_3N_2O_4$	C, N, H
40	8-azaspiro[4.5]decan-8-yl	2-naphthyl	147	MeCN	−40.6	44	$C_{25}H_{30}N_2O_4$	C, N, H
41	8-azaspiro[4.5]decan-8-yl	3-quinolinyl	105	MeCN/H_2O 5:1	−33.6	43	$C_{24}H_{29}N_3O_4$	C, H, N
42	8-azaspiro[4.5]decan-8-yl	2-indolyl	109	MeCN	−39.8	23	$C_{23}H_{29}N_3O_4$	C, H, N
43	3-azaspiro[5.5]undecan-3-yl	3-ClC_6H_4	95	MeCN	−16.3	55	$C_{22}H_{29}ClN_2O_4$	C, H, N
44	3-azaspiro[5.5]undecan-3-yl	3,4-$Cl_2C_6H_3$	107	MeCN	−25.5	52	$C_{22}H_{28}Cl_2N_2O_4$	C, H, N
45	3-azaspiro[5.5]undecan-3-yl	2-naphthyl	176	EtOH/H_2O 2:1	−36.0	57	$C_{26}H_{32}N_2O_4$	C, H, N
46	3-azaspiro[5.5]undecan-3-yl	3-quinolinyl	123	EtOH/H_2O 2:5	−31.6	50	$C_{25}H_{31}N_3O_4$	C, H, N
47	2-azaspiro[4.4]nonan-2-yl	3-ClC_6H_4	70	MeCN/H_2O 4:1	−14.7	45	$C_{20}H_{25}ClN_2O_4$	C, H, N
48	2-azaspiro[4.5]decan-2-yl	3-ClC_6H_4	89	MeCN/Et_2O 4:1	−16.8	54	$C_{21}H_{27}ClN_2O_4$	C, H, N
49	2-azaspiro[4.5]decan-2-yl	3,5-$Cl_2C_6H_3$	113	MeCN	−13.8	56	$C_{21}H_{26}Cl_2N_2O_4$	C, H, N
50	decahydroisoquinolin-2-yl	3-ClC_6H_4	84	MeCN	−24.0[b]	56	$C_{21}H_{27}ClN_2O_4$	C, H, N
51	[2-(1-adamantyl)ethyl]amino	3-ClC_6H_4	187	MeCN/MeOH 4:1	+8.4[b]	58	$C_{24}H_{31}ClN_2O_4$	C, H, N
52	[2-(1-adamantyl)ethyl]amino	3,5-$Cl_2C_6H_3$	182	MeCN/H_2O 5:1	+8.9[b]	55	$C_{24}H_{30}Cl_2N_2O_4$	C, H, N
53	spiro[5.5]undecyl-3-amino	3-ClC_6H_4	146	MeCN	−6.6	59	$C_{23}H_{31}ClN_2O_4$	C, H, N
54	spiro[5.5]undecyl-3-amino	3,5-$Cl_2C_6H_3$	158	MeCN	−6.6	57	$C_{23}H_{30}Cl_2N_2O_4$	C, H, N
55	decahydronaphthalenyl-2-amino	3-ClC_6H_4	83	*i*-PrOH/H_2O 1:2	+13.4[b]	37	$C_{22}H_{29}ClN_2O_4$	C, H, N
56[e]	(3,3-dimethylbutyl)amino	3-ClC_6H_4	131	EtOH/H_2O 1:1	−20.5[b]	50	$C_{18}H_{25}ClN_2O_4$	C, H, N
57[e]	8-azaspiro[4.5]decan-8-yl	3-ClC_6H_4	140	MeCN	+16.9	56	$C_{21}H_{27}ClN_2O_4$	C, H, N
58[e]	8-azaspiro[4.5]decan-8-yl	3,5-$Cl_2C_6H_3$	190	MeCN	+16.6	60	$C_{21}H_{26}Cl_2N_2O_4$	C, H, N
59[e]	8-azaspiro[4.5]decan-8-yl	3,4-$Cl_2C_6H_3$	151	MeCN	+23.0	66	$C_{21}H_{26}Cl_2N_2O_4$	C, H, N
60[e]	[2-(1-adamantyl)ethyl]amino	3-ClC_6H_4	187	MeCN/MeOH 4:1	−8.0[b]	58	$C_{24}H_{31}ClN_2O_4$	C, H, N
61[f]	dipentylamino	3,4-$Cl_2C_6H_3$	114	*i*-Pr ether	+17.0[g]	64	$C_{22}H_{32}Cl_2N_2O_4$	C, H, N
62[h]	dipentylamino	2-naphthyl	86	EtOH/H_2O 1:2	+11.0[g]	68	$C_{26}H_{36}N_2O_4$	C, H, N

[a] $[\alpha]^{25}$ in MeOH (3%). [b] $[\alpha]^{25}$ in $CHCl_3$ (3%). [c] Compound 16 was isolated as the calcium salt. [d] *N*: calcd, 6.97; found, 6.85. [e] Compounds 56–60 are enantiomers of the *S* series synthetized for the sake of comparison. [f] CR 1456 [(*R*)-lorglumide]: see ref 11. [g] $[\alpha]^{25}$ in EtOH (2.5%). [h] CR 1795: Rotta Patent, European Application 87830442.7 and ref 13.

though having high antigastrin activity in vivo, exhibit only a low affinity with the central CCK-B receptor in vitro.

In order to try to clarify these conflicting results, the activity of the most significant among the above-mentioned compounds was evaluated for [^{3}H]pentagastrin receptor binding to rabbit gastric glands.

This model gave us the highest specific binding and therefore was preferred to models using other species, such

Table II. Biological Activities of (*R*)-4-Benzamido-5-oxopentanoic Acid Derivatives (1–62)

compd	IC_{50},[a,c] μM CCK-A	IC_{50},[a,c] μM CCK-B (B_2)	A/B_2 ratio	ID_{50},[b,c] mg/kg
1	IN[g] (>100)	50.6 (39–65.5)		64 (55–75)
2	IN (>100)	96.0 (71–130)		70 (56–87)
3	66.5 (40–110)	30.3 (20.8–44)	2.2	36 (24–55)
4	31.3	36.2 (32.8–39.9)	0.9	49
5	8.6 (5.1–14.6)	10.2 (7.4–14)	0.8	62
6	80.0	21.5 (17.8–26)	3.7	38 (24–60)
7	IN (>100)	28.0 (14–56)		IN (>75)
8	IN (>100)	35.3 (29.5–42.2)		35 (30–42)
9	IN (>100)	48.5 (39.2–60)		40 (29–56)
10	90.5 (68–120)	10.1 (4.6–22)	9.0	16 (13–20)
11	IN (>100)	2.9 (2.1–4)		40
12	73.5	3.6 (3.1–4.2)	20.4	16 (12–22)
13	18.8 (15.8–22.2)	3.1 (1.1–8.7)	6.1	46
14	13.0 (8.2–20.6)	2.3 (1.1–4.8)	5.7	25 (19–33)
15	22.3 (17.6–28.2)	1.2 (0.2–7.7)	18.6	23 (16–34)
16	27.9	3.2 (2.1–4.9)	8.7	20 (15–26)
17	0.3 (0.1–1)	1.6 (0.7–3.5)	0.2	24 (18–32)
18	11.1 (6.2–20)	1.9 (1.6–2.3)	5.8	20 (9–45)
19	8.0 (4.8–13.4)	0.9 (0.4–2.5)	8.9	21 (16–28)
20	6.8 (3.3–14)	10.2 (6.1–17)	0.7	IN (>50)
21	80.3	20.8 (15–28.8)	3.9	36 (32–42)
22	60.0	7.0 (6.1–8)	8.6	33
23	28.9 (16.0–52.3)	1.0 (0.3–3)	28.9	40 (22–72)
24	IN (>100)	5.8 (4.8–7)		23 (20–26)
25	25.5 (19.8–32.8)	3.3 (1.8–6.2)	7.7	22 (18–28)
26	62.5 (40.9–95.5)	8.0 (4.3–15)	7.8	30 (26–35)
27	11.0 (8.6–14.1)	1.2 (0.5–2.7)	9.2	15 (13–18)
28	13.5 (10–18.2)	0.6 (0.4–0.8)	22.5	11 (8–15)
29	1.3 (0.7–2.4)	0.5 (0.2–0.9)	2.6	30 (18–51)
30	20.8 (14.1–30.6)	0.8 (0.3–2.2)	26.0	28
31	67.5	16.3 (10.3–25.7)	4.1	39
32	72.2	16.1 (12.8–20.2)	4.5	52
33	48.2	19.0 (12.8–28)	2.5	31 (17–56)
34	27.3 (22.2–33.4)	2.7 (1.2–6.1)	10.1	31 (24–40)
35	IN (>100)	16.5 (5.8–47)		35 (29–42)
36	IN (>100)	IN (>40)		67 (26–171)
37	93.5	IN (>40)		66
38	19.2	10.5 (6.2–18)	1.8	23 (15–36)
39	12.5 (8.3–18.8)	6.2 (5.3–7.3)	2.0	28 (20–39)
40	2.0 (1.3–3)	0.3 (0.1–0.5)	6.6	31 (22–44)
41	1.3 (0.8–2.2)	2.3 (0.5–11)	0.6	IN (>50)
42	3.8 (2.6–5.5)	0.4 (0.2–1.1)	9.5	33 (25–44)
43	6.3 (3.6–10.9)	0.8 (0.4–1.6)	7.9	33 (26–43)
44	0.4 (0.2–0.7)	0.4 (0.2–0.6)	1.0	IN (>50)
45	0.6 (0.4–0.9)	0.2 (0.1–0.3)	3.0	3.8
46	0.4 (0.2–0.8)	0.8 (0.4–1.5)	0.5	41
47	25.2 (15.9–40)	8.1 (5.0–13.1)	3.1	28 (23–34)
48	14.0 (9–21.7)	3.8 (3.4–4.3)	3.7	38
49	10.2 (7.5–13.8)	0.7 (0.4–1.2)	14.6	30 (23–38)
50	88.8	2.3 (1.3–4.2)	38.6	21 (12–36)
51	33.3 (27.2–40.8)	0.4 (0.3–0.6)	83.2	22 (11–44)
52	32.8 (19.0–58)	0.3 (0.1–0.8)	109.3	17 (14–21)
53	35.0	7.8 (5.5–11)	4.5	IN (>50)
54	27.3 (19.6–38.1)	6.0 (4.4–8.2)	4.6	IN (>50)
55	23.0 (17.7–29.9)	1.9 (1.1–3.4)	12.1	IN (>50)
56[d]	IN (>100)	44.0 (29–66.6)		55
57[d]	40.2	5.8 (2.4–14)	6.9	IN (>50)
58[d]	38.4 (25.3–58.3)	3.0 (1.9–4.7)	12.8	47
59[d]	10.5 (7.1–15.6)	2.3 (1.8–3)	4.6	IN (>50)
60[d]	65.8	2.8 (2–3.9)	23.5	52
61[e]	0.05 (0.03–0.09)	3.0 (1.9–4.8)	0.02	IN (>50)
62[f]	0.03 (0.02–0.05)	0.5 (0.2–1.5)	0.06	IN (>50)
CCK-8	0.5 (0.2–1.2) nM	1.0 (0.6–1.6) nM	0.5	
pentagastrin	1750 (1434–2135) nM	6.8 (5.6–8.3) nM	257.4	

[a] IC_{50}: μM displacing concentration and $p = 0.05$ fiducial limits required to inhibit by 50% the specific binding of 25 pM [^{125}I](BH)-CCK-8 in rat pancreatic acini (CCK-A) and rat brain cortex (CCK-B or B_2). [b] IC_{50}: compound dose in mg/kg iv (bolus) and $p = 0.05$ fiducial limits required to inhibit by 50% in the perfused rat stomach the acid secretion induced by 30 μg/kg per h of pentagastrin infusion. [c] Values without fiducial limits were obtained from not more than two separate tests. [d] See footnote *e* of Table I. [e] Compound **61** or (*R*)-lorglumide. [f] See footnote *h* of Table I. [g] IN = inactive.

as guinea pig or rat. The results obtained, reported in Table III, may be accounted for by the difference of species employed, but in general a good match with the results observed in vivo (on the inhibition of pentagastrin stimulated acid gastric secretion in the rat) was obtained. In fact compound **28** is the most potent gastrin inhibitor of the series in both in vivo and in vitro models; in addition, compounds **10**, **12**, and **27** display important anti-gastrin activity in vivo as well as on gastric glands in vitro.

Compound **45** and (*R*)-lorglumide, which are barely ef-

Table III. Comparison of Binding Inhibition of 4-Benzamido-5-oxopentanoic Acid Derivatives in Different Models (IC_{50}, μM)

compd	[^{125}I]CCK-8[a] rat pancreatic acini (CCK-A)	[^{125}I]CCK-8[a] rat brain cortex (CCK-B or B_2)	[^{3}H]pentagastrin:[b] rabbit gastric glands (CCK-B or B_1)	A/B_2[a] ratio	B_1/B_2 ratio
10	90.5	10.1	2.2 (1.4–3.5)	9.0	0.2
12	73.5	3.6	2.0 (1.1–3.5)	20.4	0.5
27	11.0	1.2	0.8 (0.3–2.0)	9.2	0.7
28	13.5	0.6	0.2 (0.1–0.4)	22.5	0.3
45	0.6	0.2	23.0 (15.8–33.5)	3.0	115
51	33.3	0.4	8.3 (5.7–12.0)	83.2	20.8
52	32.8	0.3	8.1 (5.4–12.2)	109.3	27.0
(*R*)-lorglumide	0.05	3.0	25.0 (18.5–33.7)	0.02	8.3
pentagastrin	1750 nM	6.8 nM	6 (4.4–8.1) nM	257.4	0.9

[a] Values drawn from Table II. [b] IC_{50}: μM displacing concentration and $p = 0.05$ fiducial limit against 6.7 nM [^{3}H]pentagastrin.

fective or totally ineffective in the inhibition of gastric secretion, exhibit, as expected, only slight activity on this in vitro model.

By examining the ratios A/B_2 and B_1/B_2 of Table III (the stomach gastrin receptor and the CCK central receptor are termed by us CCK-B_1 and CCK-B_2, respectively), it is possible to observe that compounds **10**, **12**, **27**, and **28** have the equivalent property of exhibiting 10–20 times higher affinity for the CCK-B_2 central receptor in comparison with the peripheral CCK-A receptor, whereas the affinity for the CCK-B_1 peripheral gastrin receptor is even higher than that for the central CCK-B_2, also with the same order of magnitude.

Conversely, compound **45** shows a high affinity for the central CCK-B_2 receptor as well as for the peripheral CCK-A receptor, whereas it is, as already stated, almost ineffective in inhibiting gastrin binding to the CCK-B_1 peripheral gastrin receptor (B_1/B_2 ratio is 115). Furthermore the adamantyl derivatives **51** and **52**, exhibit a much higher affinity with the CCK-B_2 receptor than that displayed on inhibiting the binding on both CCK-A and pentagastrin CCK-B_1 peripheral receptors.

Taken together, these results are further evidence of what was already hypothesized by others,[13] that at least three receptor subtypes for the CCK/gastrin system exist and that the stomach gastrin receptor (CCK-B_1) may not be so closely related to the central CCK-B (or CCK-B_2) receptor as previously hypothesized.

The presence or the lack of a second C-5 straight alkyl chain in COR_1 seems to be, therefore, a major factor in directing these 4-benzamido-5-oxopentanoic acid derivatives to antagonism with CCK-A rather than CCK-B_1 or CCK-B_2 receptors. The relative inactivity of (*R*)-lorglumide in inhibiting the pentagastrin binding on the putative CCK-B_1 receptor, together with the lack of any anti-gastrin activity in vivo, has led us to suppose that the 4-benzamido-5-oxodi-*n*-alkylpentanoic acid derivatives could compete at the receptor site with the 26–28 CCK moiety rather than with the terminal pentapeptide (29–33) of CCK. On the other hand, the 4-benzamido-5-oxopentanoic acid derivatives in which COR_1 is either a secondary amido group having a bulky substituent not less than two unsubstituted carbon units from amido group or a tertiary amido group of the optimum size of a bicyclo spiro group are able to compete especially with the terminal pentapeptide sequence shared by both CCK and gastrin. This speculation reflects the experimental results obtained with the two series of pentanoic acid derivatives, i.e. the lorglumide series and this new CCK-B antagonist series, but evidently other explanations for such binding selectivities are also possible.

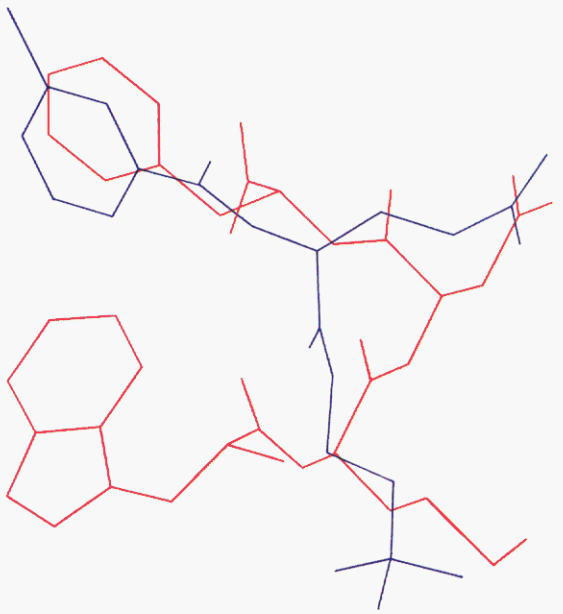

Figure 1. Computer superposition of **10** on tetragastrin CCK-(30–33) showing carboxyl group, aromatic ring, and methionine and *tert*-butyl alkyl chain matching: Red, tetragastrin; blue, compound **10**.

To further understand the structure–activity relationships between the terminal CCK tetrapeptide (Trp-Met-Asp-PheNH_2) (C30–C33) and the 4-benzamido-5-oxopentanoic acid derivatives CCK/gastrin inhibitors, a computer-assisted conformational analysis was carried out in order to determine the low-energy conformation of both the agonist and its antagonists. For the paradigmatic example the structure of compound **10** was fitted to the low-energy structure for tetragastrin, taking the carboxylic acid of both molecules as an anchor group and allowing other groups of compound **10** to orient independently within the space that the computer graphics gave approximately as the best superposition of the two structures. However, for both molecules the minimized structure energy, that is, 29.28 kcal/mol for compound **10** (coded CR 2093) and 60.23 kcal/mol for tetragastrin, was maintained.

As shown in Figure 1, it seems that once we have fixed the superposition of both carboxylic groups of the two molecules and the superposition of the 3-chlorobenzamido moiety of compound **10** with the region of space of terminal phenylalaninamide group of tetragastrin, we can see that the COR_1 4,4-dimethylbutylamide side chain of **10** exhibits

(13) Cherner, J. A.; Sutliff, V. E.; Grybowski, D. M.; Jensen, R. T.; Gardner, J. D. Functionally Distinct Receptors for Cholecystokinin and Gastrin of Dispersed Chief Cells from Guinea Pig Stomach. *Am. J. Physiol.* **1988**, *254*, G151–G155.

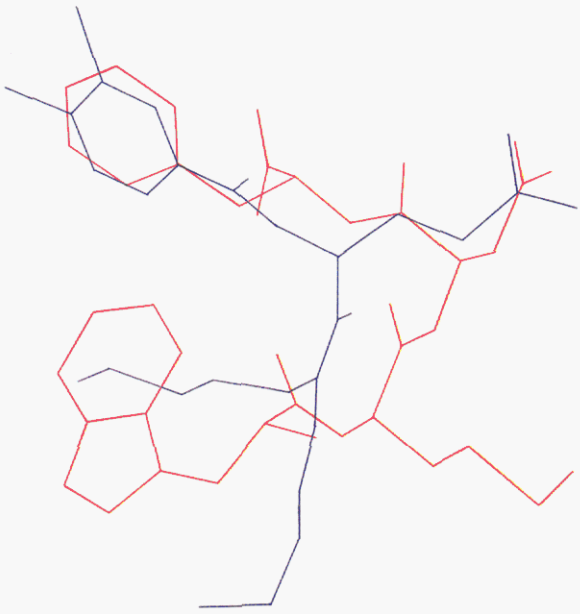

Figure 2. Computer superposition of (*R*)-lorglumide on tetragastrin CCK(30–33) showing carboxyl group and aromatic ring matching and overlay lacking between methionine and *n*-pentyl groups: red, tetragastrin; blue, (*R*)-lorglumide.

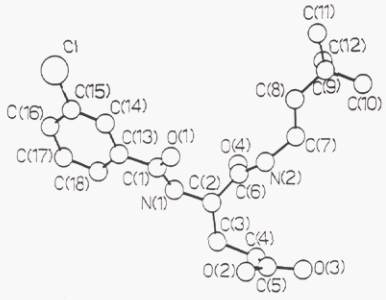

Figure 3. A perspective view of **10** showing the atomic numbering scheme.

an extensive overlap with the hydrophobic methionine moiety. One might therefore speculate that R_1 could serve as an additional anchor group for interaction with a second hydrophobic region of the gastrin receptor. In contrast, the superpositioning of (*R*)-lorglumide with CCK(30–33) by matching both the carboxyl group and the phenyl ring of the two structures does not allow to successfully overlay the residual hydrophobic moieties, i.e. the methionine and *n*-pentyl groups (Figure 2).

To validate the results of computer conformational analysis, an X-ray structure study of compounds **10** and (*R*)-lorglumide was performed. In Figure 3 an arbitrary projection of the molecule of compound **10** with the atomic numbering scheme is shown.

A small deviation from planarity is present in the chlorobenzamido group (plane I) where C14 lies 0.02 Å from the least-squares plane. No significant deviation from planarity is detected on the C1, N1, O1, C13 group (plane II), while the group C6, O4, N2, C2 shows a small distortion from planarity (plane III).

Planes I and II form an angle of 17.35°, while planes II and III form an angle of 74.73°. A gauche conformation is present along the sequence C2–C3–C4–C5 (torsion angle of 64.7°). All packing distances are within the expected range and bond angles; bond lengths and torsion angles selected to describe the geometry of the molecule by the X-ray method do not differ significantly from those calculated by the computer conformational analysis and used above in superposition drawings.

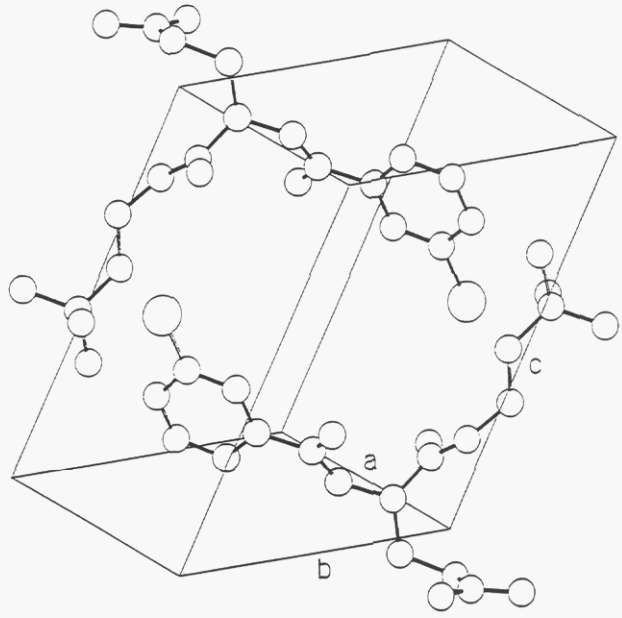

Figure 4. Molecular packing in the triclinic unit cell of **10**.

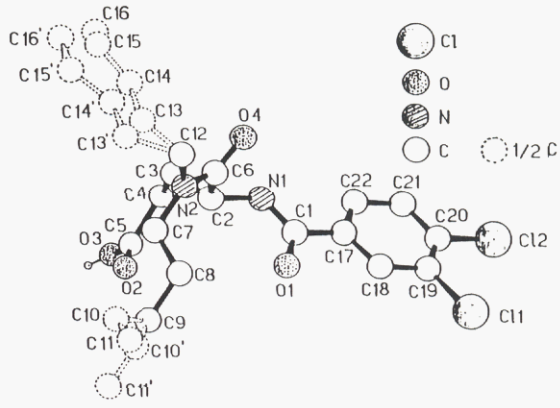

Figure 5. A perspective view of (*R*)-lorglumide showing the atomic numbering scheme. Dashed atoms belong to disordered moieties.

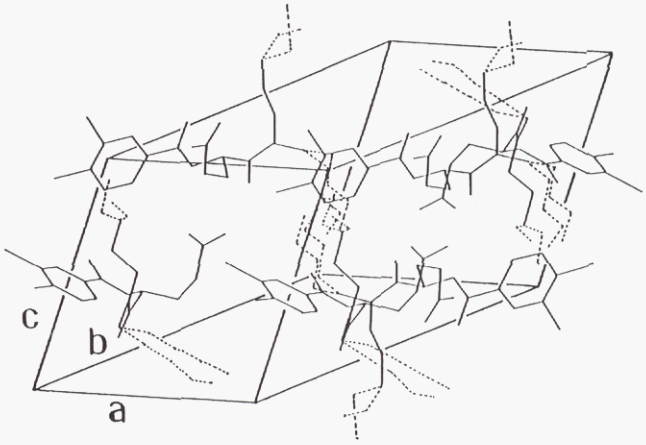

Figure 6. Molecular packing in the monoclinic unit cell of (*R*)-lorglumide. Dashed lines show the disordered *n*-pentyl sequences.

The molecular packing diagram of compound **10** in the unit cell is illustrated in Figure 4.

Figure 5 shows an arbitrary projection of the molecule of (*R*)-lorglumide where disorder affecting the two pentyl

Table IV. Comparison of Antigastrinic Activity of (*R*)-4-(3,5-Dichlorobenzamido)-5-(8-azaspiro[4.5]decan-8-yl)-5-oxopentanoic Acid (Compound **28**) in Different Models in Vivo

	pentagastrin dose, μg/kg per h	ID_{50},[a] mg/kg (fiducial limits) 0–60 min	60–120 min	route of administration
perfused rat stomach[b]	30	11.0 (8–15)		iv (bolus)
secretion in gastric fistula cat model	6	15.5 (8–30)	44.2 (24–85)	iv (bolus)
secretion in gastric fistula dog model	3	8.7 (4–19)	28.8 (16–50)	iv (bolus)
secretion in Heidenhain pouch dog model	6	24.2 (19–31)	33.6 (24–46)	oral

[a] ID_{50}: compound dose in mg/kg and $p = 0.05$ fiducial limits required to inhibit by 50% the acid secretion induced by the given dose of pentagastrin infusion in the different conscious animals. For protocol, see in vivo evaluation in the Experimental Section under Biological Methods. [b] Value drawn from Table II.

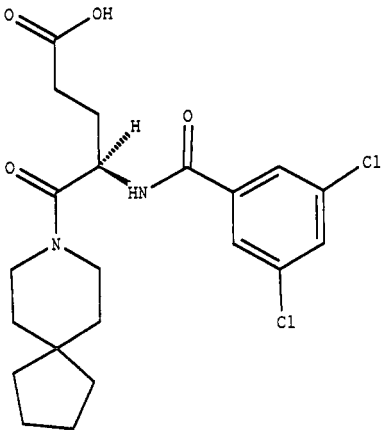

Figure 7. Compound **28** (CR 2194).

aliphatic sequences is noted. No significant deviation from planarity is detected in the dichlorobenzoyl group (plane I) nor in the C1, N1, O1, C17 (plane II) and C6, O4, N2, C2, (plane III) groups. Planes I and II form an angle of 69° while planes II and III form an angle of 97.4°.

In Figure 6 part of the crystal packing of (*R*)-lorglumide is shown, supporting the statement that disorder in the alkylic sequences is due to the poor interaction between these moieties and neighboring groups.

It is worthy to note that in the lorglumide structure the carbon atoms (C11–C16) of the two pentyl chains, which are mainly in all-trans state, are about 9 Å apart, so that the overall shape of the molecule is strongly characterized by the presence of three distinct hydrophobic moieties. This seems to be the main difference with the molecular structure of compound **10**, in which the presence of only two hydrophobic moieties of suitable dimensions, apart from the carboxylic group, seems to be more favorable for the interaction with the gastrin receptor.

Compound **28** (Figure 7), the most potent CCK/gastrin receptor antagonist of this series, was chosen for further evaluation of its anti-gastrin activity in vivo on two other animal species.

In dogs with chronic gastric fistulas, the compound was given by iv bolus in a dose range of 5–30 mg/kg, against a fixed submaximal stimulation of gastric acid secretion induced by a 2-h iv infusion of pentagastrin (3 μg/kg per h). Compound **28** dose dependently antagonizes the gastric acid output induced by pentagastrin infusion, with an ED_{50} of 8.7 mg/kg in the first hour after bolus administration and an ED_{50} of 28.8 mg/kg in the second hour.

From the above-mentioned results it seems likely that compound **28** has the same antigastrin potency in both rat and dog. In this connection, it is interesting to note that the most potent nonpeptidic CCK-B antagonist discovered up to now (i.e. the benzodiazepine derivatives L-365,260) was reported to be much less effective in the dog, that in other tested animals species, such as the mouse, rat, and guinea-pig.[9] These results lead us to suppose a qualitative difference between the two antigastrin series.

In the Heidenhein pouch dog model, the compound, given in a dose range of 10–40 mg/kg intragastrically, dose dependently reduces the gastric acid output induced by a 2-h iv infusion of 6 μg/kg per h of pentagastrin. At 40 mg/kg the reduction is about 70% in both the first and second hour collection period after its oral administration. The calculated ID_{50} are in this case 24.2 and 33.6 mg/kg, respectively.

In cats with chronic gastric fistulas the compound exhibits the same pattern of activity as in the dog. Under a 2-h 6 μg/kg per h iv pentagastrin stimulation, the gastric acid output is reduced by 50% at the dose of 15 mg/kg in the first hour after iv bolus and at the dose of 44 mg/kg in the second hour collection period.

The results obtained are shown in Figure 8 and in Table IV.

Conclusions

Compound **28** (coded CR 2194) is the most potent CCK-B antagonist among the newly described class of

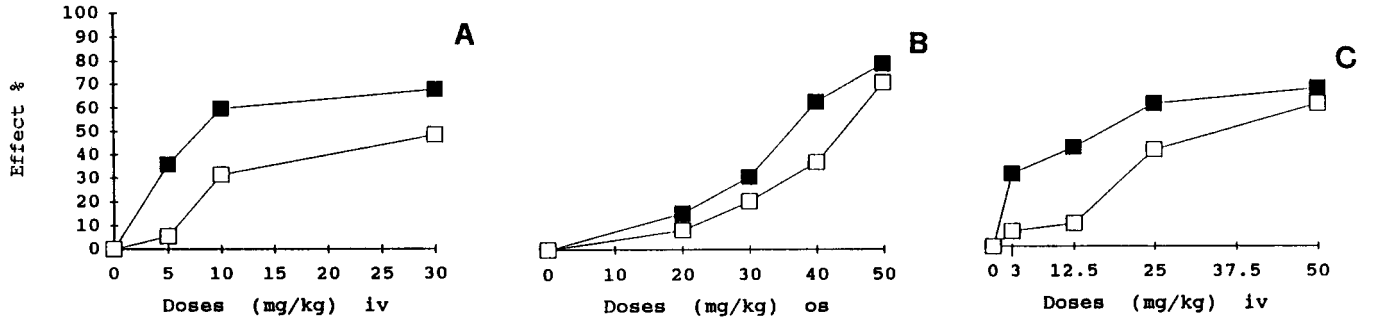

Figure 8. Effects of **28** (CR 2194) on dose–response curves in antagonizing gastrin-stimulated acid secretion in different models: (A) dog with gastric fistula; pentagastrin infusion dose of 3 μg/kg per h; (B) dog with Heidenhain pouch; pentagastrin infusion dose of 6 μg/kg per h; (C) cat with gastric fistula; pentagastrin infusion dose 6 μg/kg per h. In abscissa given doses (bolus) of **28** by different routes of administration. Each point represents the mean of three different experiments. ■: Effects in the first hour (0–60 min) from bolus administration of **28**. □: Effects in the second hour (60–120 min) from bolus administration of **28**.

4-benzamido-5-oxopentanoic acid derivatives by its ability to block pentagastrin-stimulated acid secretion in several models. It has a simple nonpeptidic molecular structure and it is pharmacologically effective even after oral administration. These properties indicate that this compound could be a useful tool in the study of the biological effects of gastrin and a potential agent for diagnostic or therapeutic uses in man.

Experimental Section

Biological Tests. Male adult Sprague–Dawley rats, New Zealand white rabbits, beagle dogs, and tabby cats were used. CCK-8 and pentagastrin were purchased from Peninsula Laboratories, [^{125}I](BH)-CCK-8 (specific activity 2000 Ci/mmol) was from Amersham, Ultima Gold was from Packard, [^{3}H]pentagastrin (specific activity 31 Ci/mmol) was from NEN, rabbit serum albumin and bovine serum albumin (BSA) were from Merck (Darmstadt, Germany), bacitracin was from Serva (Heidelberg, Germany), soybean trypsin inhibitor (SBTI) was from PL Biochemicals (St. Goar, Germany), crude collagenase was from Boehringer (Mannheim, Germany), and Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], EGTA [ethylenebis(oxyethylenenitrilo)tetraacetic acid], and DTT (DL-dithiothreitol) were from Sigma.

The compounds under investigation were dissolved as sodium salt in distilled water for the in vitro studies, or in saline for the in vivo studies. Estimated concentrations or doses at 50% effect (IC_{50} or ID_{50}) and their $p = 0.05$ fiducial limits were calculated from the regression line of the percentage of maximum effect, discarding the 6% tails, on the logarithm of the concentration or of the dose.

(1) Antisecretory Activity in Rats. Fasted (24 h) male rats anesthetized with urethane were used. Gastric acid secretion was determined in the perfused rat stomach according to the method of Ghosh and Schild[14] with slight modifications. The substances under investigation were administered by iv bolus in triplicate, in at least three doses after 60 min from the beginning of pentagastrin infusion. The inhibition (%) of gastric acid secretion is calculated from the values of total acid output collected before (first 60 min of pentagastrin infusion) and after the administration of the substances (second 60 min of pentagastrin infusion).

(2) Antisecretory Activity in Dogs with a Heidenhain Pouch. Fasted (24 h) conscious Heidenhain pouched dogs[15] were used. Gastric acid secretion was stimulated by pentagastrin continuous infusion at a dose of 6 μg/kg per h into the cefalic vein. Gastric juice was collected for 2 h, at 15-min intervals following the start of the pentagastrin infusion. Total acid output was measured by titrating 1 mL of gastric juice to pH 7 with 0.1 N NaOH. Compound **28** at four dose levels and in triplicate ($n = 12$) or placebo was administered orally, 30 min before pentagastrin infusion. Total 15-min HCl output was plotted against the time of infusion; the area under the curve (AUC) was calculated and the gastric acid antisecretory activity of the compound was calculated as the percentage of the AUC of the control dogs.

(3) Antisecretory Activity in Dogs with Gastric Fistula. Conscious dogs with a gastric fistula[16] were used. The method of gastric acid stimulation is the same as that described above, with the exception of the dose of pentagastrin, which was 3 μg/kg per h, while the period of collection was 3 h. Compound **28** at three dose levels and in triplicate ($n = 9$) or saline was given intravenously 45 min following the start of the pentagastrin infusion, when the gastric acid stimulation had reached maximal levels. Total 15-min HCl output was plotted against the time of infusion; the area under the curve after drug administration was calculated and the gastric acid antisecretory activity of the compound was calculated as the percentage of the AUC of the control dogs.

(4) Antisecretory Activity in Cats with Gastric Fistula. Conscious cats with a gastric fistula were used after 24-h fasting. The method of the gastric acid stimulation is the same as described above in 2. Gastric juice was collected for 3 h, at 10-min intervals following the start of the pentagastrin infusion. Compound **28** at four dose levels and in triplicate ($n = 12$) or saline was given intravenously 40 min following the start of pentagastrin infusion, when the gastric acid stimulation had reached maximum levels. Total 10-min HCl output was plotted against the time of infusion; the area under the curve after drug administration was calculated and the gastric acid antisecretory activity of the compounds was calculated as the percentage of the AUC of the control cats.

Binding Studies. CCK-A receptor binding assays were carried out on rat pancreas by the collagenase method previously described.[17] Briefly, tissue (about 5 g) was extensively minced and dispersed in 30 vol of Krebs–Henseleit buffer (KHB) (118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM NaH_2PO_4, 0.1 mM $CaCl_2$, 14 mM glucose, and 0.1 mg/mL SBTI 1% BSA) adjusted to pH 7.4. To this medium, continuously shaken and gassed with 95% O_2–5% CO_2, was added 0.5 mg/mL crude collagenase. The resulting suspension was filtered with nylon mesh (320 μm), layered over KHB containing 4% BSA, 0.5 mM $CaCl_2$, and 0.1 mg/mL SBTI, and centrifuged for 5 min at 132g. The final resulting pellet was suspended in 10 vol of Hepes–Ringer pH 7.4 buffer (118 mM NaCl, 10 mM Hepes, 1.13 mM $MgCl_2$, 1.28 mM $CaCl_2$, and 1% BSA). This preparation gave a final concentration of $1–3 \times 10^7$ cells/mL (assessed by light microscope with the aid of a Neuebauer chamber), which was diluted with binding assay to about 5×10^6 cells/mL.

CCK-B receptor binding assays were carried out on membranes of rat cerebral cortex, as previously described.[17] The tissue (about 800 mg) was homogenized in ice-cold 20 mM Hepes (pH 6.5) and centrifuged twice at 50000g. The washed final pellet was resuspended in 50 vol of binding assay buffer (20 mM Hepes, 5 mM $MgCl_2$, 360 mM NaCl, 15 mM KCl, 1 mM EGTA, and 0.2 mg/mL bacitracin). Protein concentration was determined by using the method of Bradford,[18] using BSA as the standard. This procedure gave about 1 μg of protein/μL. Pentagastrin (CCK-B_1) receptor binding assays were carried out on isolated glands from the rabbit gastric mucosa according to the method of Berglindh[19] with minor modifications. Briefly, the fundic mucosa was separated from muscular and submucosal layers, weighed (ca. 6 g), and then minced into small pieces and transferred to 50 mL of collagenase solution (130 mM NaCl, 12 mM $NaHCO_3$, 3 mM NaH_2PO_4, 3 mM Na_2HPO_4, 3 mM K_2HPO_4, 2 mM $MgSO_4$, 1 mM $CaCl_2$, 10 mg/L phenol red, 0.2% glucose, and 1% rabbit serum albumin, pH 7.4) containing 1 mg/mL of collegenase.

The suspension was continuously gassed with 100% O_2 at 37 °C, gently stirred for 20–25 min, and then filtered through nylon mesh (320 μm). Free cells were removed by gravity sedimentation for 15 min and the glands were washed free from isolated cells and collagenase three times with buffer binding solution (132 mM NaCl, 5.4 mM KCl, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 0.2% rabbit serum albumin, 0.2% glucose, and 0.5 mM DTT, pH 7.4). This procedure gave about 7–8 mL of sedimentated glands with 90% viability (Trypan blue exclusion method). The suspension was diluted in buffer binding to obtain about 375 mg wet weight/mL.

Binding conditions for both CCK-A and CCK-B_2 binding assays were the same. Cortical membranes or acini (400 μL), tracer (55000 dpm/tube), and displacing agents were incubated in a 0.5-mL total volume in polypropylene tubes for 30 min at 37 °C.

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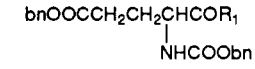
Table V. Physical Properties of Compounds **63a–y** Prepared by Path 1, Scheme I



compd	R_1	mp, °C	recryst solvent	R_f[a]	formula	anal.
63a	butylamino	124	EtOH/*i*-Pr_2O 1:3	0.38	$C_{24}H_{30}N_2O_5$	C, H, N
63b	(3-methylbutyl)amino	143	*i*-PrOH/*i*-Pr_2O 1:3	0.50	$C_{25}H_{32}N_2O_5$	C, H, N
63c	(3,3-dimethylbutyl)amino	106	EtOH/H_2O 2:1	0.78[b]	$C_{26}H_{34}N_2O_5$	C, H, N
63d	pentylamino	108	*i*-PrOH/*i*-Pr_2O 1:4	0.42	$C_{25}H_{32}N_2O_5$	C, H, N
63e	(4,4-dimethylpentyl)amino	WS[c]		0.83[b]	$C_{27}H_{36}N_2O_5$	
63f	(3-ethyl-3-methylpentyl)amino	WS[c]		0.88[b]	$C_{28}H_{38}N_2O_5$	
63g	(3,3-diethylpentyl)amino	oil		0.91[b]	$C_{29}H_{40}N_2O_5$	
63h	hexylamino	101	*i*-PrOH/*i*-Pr_2O 1:4	0.46	$C_{26}H_{34}N_2O_5$	C, H, N
63i	(2-ethylhexyl)amino	110	*i*-PrOH/*i*-Pr_2O 1:4	0.71	$C_{28}H_{38}N_2O_5$	C, H, N
63j	heptylamino	100	*i*-PrOH/*i*-Pr_2O 1:4	0.51	$C_{27}H_{36}N_2O_5$	C, H, N
63k	(4,4-dimethylcyclohexyl)amino	84	*i*-Pr_2O	0.57	$C_{28}H_{36}N_2O_5$	C, H, N
63l	(4,4-diethylcyclohexyl)amino	oil		0.58	$C_{30}H_{40}N_2O_5$	
63m	cycloheptylamino	153	EtOH/*i*-Pr_2O 1:1	0.49[e]	$C_{27}H_{34}N_2O_5$	C, H, N
63n	cyclooctylamino	120	*i*-PrOH/*i*-Pr_2O 4:1	0.48	$C_{28}H_{36}N_2O_5$	C, H, N
63o	cyclodecylamino	81	*i*-Pr_2O	0.70	$C_{30}H_{40}N_2O_5$	C, H, N
63p	decahydronaphthalen-2-ylamino	123	*i*-PrOH/*i*-Pr_2O 1:3	0.65	$C_{30}H_{38}N_2O_5$	C, H, N
63q	spiro[5.5]undecyl-3-amino	oil		0.66	$C_{31}H_{40}N_2O_5$	
63r	[2-(1-adamantyl)ethyl]amino	77	EtOH/*i*-Pr_2O 1:1	0.66	$C_{32}H_{40}N_2O_5$	C, H, N
63s	4,4-dimethylpiperidin-1-yl	oil		0.51	$C_{27}H_{34}N_2O_5$	
63t	decahydroisoquinolin-2-yl	oil		0.48	$C_{29}H_{36}N_2O_5$	
63u	2-azaspiro[4.4]nonan-2-yl	oil		0.39	$C_{28}H_{34}N_2O_5$	
63v	2-azaspiro[4.5]decan-2-yl	oil		0.41	$C_{29}H_{36}N_2O_5$	
63w	8-azaspiro[4.5]decan-8-yl	oil		0.59	$C_{29}H_{36}N_2O_5$	
63x	3-azaspiro[5.5]undecan-3-yl	oil		0.54	$C_{30}H_{38}N_2O_5$	
63y	dipentylamino	oil		0.79[d]	$C_{30}H_{42}N_2O_5$	

[a] Benzene/ethyl acetate 7:3. [b] Methylene chloride/ethanol 9:1. [c] WS: waxy solid. [d] Chloroform/ethyl acetate 9:1.

Then 1 mL of ice-cold assay buffer was added, and the tubes were centrifuged at 12500*g*. The supernatant was eliminated and the radioactivity associated with the pellet measured in a Packard 5000 γ-counter (80% efficiency). Nonspecific binding was estimated as 5 μM CCK-8 (on average, 30% for CCK-A and 45% for CCK-B_2 binding studies).

Binding conditions for pentagastrin binding assay were similar except that 220 μL of gastric glands suspension (about 0.5 mg protein/tube) was incubated with tracer (115000 dpm/tube) and displacing agent for 15 min at 37 °C in a final volume of 0.25 mL. The centrifuged pellet was digested in 1 mL of 1 N NaOH and the radioactivity measured in a Packard Tri-Carb liquid scintillator spectrometer after dilution with Ultima Gold (40% efficiency). Nonspecific binding was estimated with 0.1 mM pentagastrin (on average 50%).

Chemistry. The following procedures were adopted: ^{1}H NMR spectra were recorded at 60 MHz on a Varian EM360L or at 300 MHz on a Bruker CXP-300 instrument; infrared spectra were recorded on a Perkin-Elmer 1420 Ratio Recording IR spectrophotometer with 3700 Data Station. Melting points were determined on a Buchi 535 apparatus and are uncorrected. Elemental analysis were performed by Redox (Cologno Monzese, MI), and the analytical results were within ±0.4% of the theoretical values unless otherwise noted. TLC was carried out using Merck silica gel GF_{254} plates with the following elution systems: A (1-butanol/acetic acid/water 5:2:2), B (isoamyl alcohol/acetone/water 5:2:1), C (methylene chloride/ethanol 9:1), and D (benzene/ethyl acetate 7:3). Specific rotation was determined with a JASCO DIP-370 polarimeter at 589 nm in a 100-mm cell and at 3 g/100 mL, except where otherwise indicated. The optical purity of the enantiomers was tested by HPLC using a Varian 5500 liquid chromatograph, a Resolvosil BSA-7 column (Macherey-Nagel) as stationary phase, and 0.1 M pH 8.0 phosphate buffer + 5% *n*-PrOH as mobile phase. Butylamine, 3-methylbutylamine, 3,3-dimethylbutylamine, pentylamine, hexylamine, heptylamine, 2-ethylhexylamine, cycloheptylamine, cycloethylamine, cyclodecylamine, decahydroisoquinoline, 3-azaspiro[5.5]undecane, and dipentylamine were purchased from commercial suppliers and were used without further purifications. 4,4-Dimethylpentylamine,[20] 3-methylethylpentylamine,[21] 3,3-diethylpentylamine,[21] 4,4-dimethylcyclohexylamine,[22] 4,4-diethylcyclohexylamine,[22] cyclodecylamine,[23] 4,4-dimethylpiperidine,[24] 8-azaspiro[4.5]decane,[25] 2-azaspiro[4.4]nonane,[25] 2-azaspiro[4.5]decane,[25] 2-(1′-adamantyl)ethylamine,[26] 3-spiro[5.5]undecylamine,[22] 2-decahydronaphthylamine,[27] and N-Cbz-γ-benzyl-D-glutamic acid[28] were prepared by the cited literature methods. Acyl chlorides were synthesized from the corresponding commercially available acids by refluxing with thionyl chloride and were purified according to conventional methods.

(*R*)-4-[(Carbobenzyloxy)amino]-5-[(3,3-dimethylbutyl)amino]-5-oxopentanoic Acid, Benzyl Ester (63c). To a mechanically stirred solution of 10 g (26.93 mmol) D-*N*-(carbobenzyloxy)(Cbz)glutamic acid 5-benzyl ester and 3.83 mL (27.46 mmol) of triethylamine in 50 mL of THF at −10 °C was added dropwise 2.62 mL (27.46 mmol) of ethyl chloroformate. Stirring was continued at −10 °C for 15 min and then 3.27 g (32.31 mmol) of 3,3-dimethylbutylamine was added dropwise at the same temperature. At the end the stirring was continued for 1 h at −10 °C and for 3 h at room temperature. After removal of white solid by filtration, evaporation of the solvent under reduced pressure left a crude product, an AcOEt solution which was washed

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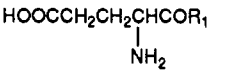
Table VI. Physical Properties of Compounds **64a–y** Prepared by Path 2, Scheme I



compd	R_1	mp, °C	recryst solvent	$[\alpha]^{25}_D$,[a] deg	formula	anal.
64a	butylamino	148	Me_2CO/H_2O 3:1	−14.2	$C_9H_{18}N_2O_3$	C, H, N
64b	(3-methylbutyl)amino	148	Me_2CO/H_2O 4:1	−21.9[b]	$C_{10}H_{20}N_2O_3$	C, H, N
64c	(3,3-dimethylbutyl)amino	154	H_2O	−25.9[b]	$C_{11}H_{22}N_2O_3$	C, H, N
64d	pentylamino	138	Me_2CO/H_2O 5:1	−9.2	$C_{10}H_{20}N_2O_3$	C, H, N
64e	(4,4-dimethylpentyl)amino	WS[c]			$C_{12}H_{24}N_2O_3$	
64f	(3-ethyl-3-methylpentyl)amino	126	Me_2CO/H_2O 4:1	−16.7[b]	$C_{13}H_{26}N_2O_3$	C, H, N
64g	(3,3-diethylpentyl)amino	117	Me_2CO/H_2O 4:1	−17.1[b]	$C_{14}H_{28}N_2O_3$	C, H, N
64h	hexylamino	135	Me_2CO/H_2O 4:1	−9.3	$C_{11}H_{22}N_2O_3$	C, H, N
64i	(2-ethylhexyl)amino	oil			$C_{13}H_{26}N_2O_3$	
64j	heptylamino	136	Me_2CO/H_2O 3:1	−9.0	$C_{12}H_{24}N_2O_3$	C, H, N
64k	(4,4-dimethylcyclohexyl)amino	183	Me_2CO/H_2O 1:1	−13.6	$C_{13}H_{24}N_2O_3$	C, H, N
64l	(4,4-diethylcyclohexyl)amino	157	H_2O	−11.1	$C_{15}H_{28}N_2O_3$	C, H, N
64m	cycloheptylamino	169	Me_2CO/H_2O 1:1	−15.4	$C_{12}H_{22}N_2O_3$	
64n	cyclooctylamino	166	H_2O	−16.0	$C_{13}H_{24}N_2O_3$	C, H, N
64o	cyclodecylamino	155	Me_2CO/H_2O 4:1	−19.6[b]	$C_{15}H_{28}N_2O_3$	C, H, N
64p	decahydronaphthalen-2-ylamino	159	$MeCO/H_2O$ 3:1	−17.6[b]	$C_{15}H_{26}N_2O_3$	C, H, N
64q	spiro[5.5]undec-3-ylamino	143	H_2O	−11.7	$C_{16}H_{28}N_2O_3$	C, H, N
64r	[2-(1-adamantyl)ethyl]amino	170	H_2O	−7.3	$C_{17}H_{28}N_2O_3$	C, H, N
64s	4,4-dimethylpiperidin-1-yl	159	Me_2CO/H_2O 2:1	−17.2	$C_{12}H_{22}N_2O_3$	C, H, N
64t	decahydroisoquinolin-2-yl	161	H_2O	−8.6	$C_{14}H_{24}N_2O_3$	C, H, N
64u	2-azaspiro[4.4]nonan-2-yl	131	Me_2CO/H_2O 4:1	−8.3	$C_{13}H_{22}N_2O_3$	C, H, N
64v	2-azaspiro[4.5]decan-2-yl	137	Me_2CO/H_2O 4:1	−5.6	$C_{14}H_{24}N_2O_3$	C, H, N
64w	8-azaspiro[4.5]decan-8-yl	175	Me_2CO/H_2O 3:1	−10.0	$C_{14}H_{24}N_2O_3$	C, H, N
64x	3-azaspiro[5.5]undecan-3-yl	170	Me_2CO/H_2O 1:1	−11.2	$C_{15}H_{26}N_2O_3$	C, H, N
64y	dipentylamino	oil			$C_{15}H_{30}N_2O_3$	

[a] c = 3, 2 N NaOH. [b] c = 3, MeOH. [c] WS: waxy solid.

with 2 N HCl (50 mL) and water to remove excess unreacted amine. The organic layer was then washed with cold 0.1 N NaOH (50 mL) and water. The solvent was dried and removed under reduced pressure and the residue was precipitated with diisopropyl ether, filtered, and dried to give 9.67 g (79%) of **63c**. An analytical sample was obtained by recrystallization from ethanol/water 2:1: mp 106 °C; TLC C (R_f 0.78); ^{1}H NMR ($CDCl_3$) 0.9 (s, 9 H, *t*-Bu), 1.15–1.65 (m, 2 H, CH_2-*t*-Bu), 1.85–2.3 (m, 2 H, C(N-Cbz)CH_2), 2.3–2.7 (m, 2 H, CH_2COObn), 3–3.5 (m, 2 H, NCH_2), 4.1–4.65 (m, 1 H, CHN), 5.1 (s, 4 H, CH_2Ph), 6.2 (d, 1 H, CNHCOObn, J = 8 Hz), 6.6–7 (m, 1 H, CONH), 7.35 ppm (s, 10 H, Ph); IR (KBr) 3303, 3051, 2956, 1739, 1693, 1649, 1540 cm^{-1}; $[\alpha]^{25}_D$ = +8.04° (c = 3; $CHCl_3$). Anal. ($C_{26}H_{34}N_2O_5$) C, H, N. With this procedure compounds **63a–y**, shown in Table V, were synthesized.

(*R*)-4-Amino-5-[(3,3-dimethylbutyl)amino]-5-oxopentanoic Acid (64c). A solution of 8 g (17.6 mmol) of **63c** in 60 mL of methanol and 10 mL of water containing 0.2 g of 10% palladium on activated charcoal was stirred for 2 h under hydrogen at atmospheric pressure and room temperature. The solution was filtered and evaporated to dryness under reduced pressure. The solid crude product was treated with acetone and collected on a filter (3.3 g, 81% yield). An analytical sample was obtained with a crystallization from water: mp 154 °C; TLC A (R_f 0.70); ^{1}H NMR (dilute NaOD) 0.9 (s, 9 H, *t*-Bu), 1.15–1.65 (m, 2 H, CH_2-*t*-Bu), 1.65–2 (m, 2 H, $CNCH_2$), 2–2.55 (m, 2 H, CH_2COOH), 2.9–3.6 ppm (m, 3 H, CH and NCH_2); IR (KBr) 3394, 3249, 3068, 2955, 1683, 1555, 1397, 1265 cm^{-1}; $[\alpha]^{25}_D$ = −25.9° (c = 3; MeOH). Anal. ($C_{11}H_{22}N_2O_3$) C, H, N.

With this procedure the compounds **64a–y**, shown in Table VI, were synthesized.

(*R*)-4-(3-Chlorobenzamido)-5-[(3,3-dimethylbutyl)-amino]-5-oxopentanoic Acid (Compound 10 or CR 2093). To a vigorously stirred solution of 3 g (13.03 mmol) of **64c** and 13.68 mL of 1 N NaOH in 15 mL of water and 20 mL of THF at room temperature were added simultaneously from two dropping funnels 13.68 mL of 1 N NaOH and 1.75 mL (13.68 mmol) of 3-chlorobenzoyl chloride dissolved in 20 mL of THF. Then stirring was continued for 3 h and finally, after partial removal of the THF under vacuum, the solution was acidified to Congo red with dilute HCl. The resulting oil was extracted with AcOEt; the organic layer was selectively extracted with 0.1 N NaOH until the AcOEt was free from 3-chlorobenzoic acid. The solvent was dried and evaporated to dryness, resulting in a viscous oil which solidified under petroleum ether. The white solid was filtered to give 3.8 g (80% yield). An analytical sample was obtained by a recrystallization from ethanol/water 1:1: mp 132 °C; TLC B (R_f 0.73); ^{1}H NMR (DMSO-d_6) 0.9 (9 H, s, *t*-Bu), 1.2–1.6 (2 H, m, CH_2-*t*-Bu), 0.9 (9 H, s, *t*-Bu), 1.2–1.6 (2 H, m, CH_2-*t*-Bu), 1.8–2.6 (4 H, m, CH_2CH_2COOH), 2.9–3.4 (2 H, m, NCH_2), 4.2–4.7 (1 H, m, CH), 7.5–8.1 (5 H, m, C_6H_4 and NH of 3,3-dimethylbutylamide), 8.6 (1 H, d, J = 8 Hz, NHCOPh, exchangeable), 12.1 ppm (1 H, s, COOH, exchangeable); IR (KBr) 3291, 3094, 2963, 1732, 1633, 1573, 1547, 1204, 733 cm^{-1}; $[\alpha]^{25}_D$ = +21.75° ($CHCl_3$); optical purity evaluated by HPLC was higher than 99%, t_R 7.9 min (t_R of *S* enantiomer of **10**, i.e. compound **56** is 7.0 min). Anal. ($C_{18}H_{25}ClN_2O_4$) C, H, N.

With this procedure compounds **1–62** shown in Table I, were synthesized.

Table VII. Crystal Data for Compound **10** and (*R*)-Lorglumide

	10	(*R*)-lorglumide
formula	$C_{18}H_{25}ClN_2O_4$	$C_{22}H_{32}Cl_2N_2O_4$
crystal dimensions (mm)	0.3 × 0.3 × 0.5	0.5 × 0.5 × 0.2
crystal system	triclinic	monoclinic
space group	$P1$	$P2_1/c$
a, Å	9.148 (2)	12.418 (7)
b, Å	10.685 (2)	18.786 (4)
c, Å	11.347 (2)	12.598 (6)
α	107.67° (1)	
β	96.51° (1)	120.2 (4)
γ	108.37° (1)	
V, Å^3	975	2540
Z[a]	2	4
density calcd, g/cm^3	1.261	1.206
intensities (unique)	4522	4731
intensities >3$\sigma(I)$	2131	1964
R[b]	6.5	8.9
R_w[b]	6.7	9.8

[a] Number of formula units in the unit cell. [b] Disagreement factors for observed reflections.

Computer-Aided Design. The software used for superimposition of **10** and (*R*)-lorglumide on tetragastrin CCK (30–33) was MAD (Molecular Advanced Design) (available from Aquitaine Systemes, Tour ELF, La Defense, Paris, France) and run on an IBM 6150 with the graphics station IBM 5085. The optimization of low-energy conforms was conducted by Monte Carlo–Metropolis

algorithm, using a dynamic weighting of the randomly chosen rotation axes to be modified. The starting data set is minimized using a function that randomly selects the rotation axes. During the first part of the calculation, the "heaviest" rotation axes are favored, next the "lightest" ones (second part), and finally the "heaviest" ones again (third part). One hundred iteractions with maximums or dE less than 0.1 kcal/mol were performed for optimization, according to Newton-Raphson.

Crystal Analysis of 10. A colorless crystal suitable for X-ray diffraction studies for both compound **10** and (*R*)-lorglumide, prepared by slow cooling of an ethyl acetate solution, was mounted on an ENRAF-NONIUS CAD-4 diffractometer and irradiated with monochromatized Mo Kα radiation using the $\theta/2\theta$ scan technique. The intensities were correlated for Lorentz and polarization factors. The structures were solved by direct methods with MULTAN-80[29] and refined with SHELX-76[30] using blocked full-matrix least-square refinement. All non-hydrogen atoms were refined anisotropically, while hydrogen atoms, located by different Fourier maps or by theoretical calculations, were refined isotropically. The main crystal data obtained for both compound **10** and (*R*)-lorglumide are registered in Table VII.

Supplementary Material Available: Tables containing the atomic coordinates, bond distances, bond angles, torsion angles, and weighted least-square planes through the selected atoms from X-ray diffraction studies for compound **10** (coded CR 2093) and (*R*)-lorglumide, tables containing the atomic coordinates, bond distances, bond angles, and torsion angles from computer-aided design for compound **10**, (*R*)-lorglumide, and tetragastrin (39 pages). Ordering information is given on any current masthead page.

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Synthesis, Characterization, and Biological Evaluation of a Novel Class of *N*-(Arylethyl)-*N*-alkyl-2-(1-pyrrolidinyl)ethylamines: Structural Requirements and Binding Affinity at the σ Receptor

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By synthesizing and testing a part-structure, *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (**3**), derived from our previously reported high affinity σ receptor ligands (1*S*,2*R*)-(−)-*N*-[2-(3,4-dichlorophenyl)-ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine [(−)-**2**] and (+)-**2**, we have identified a novel class of superpotent (subnanomolar affinity) σ ligands specific for the σ receptor labeled by [^{3}H]-(+)-3-PPP. When **3** was tested for its capacity to displace [^{3}H]-(+)-3-PPP from guinea pig brain membranes, it exhibited a K_i of 0.34 nM, which is better than either of its parent compounds (−)-**2** (K_i = 1.3 nM) and (+)-**2** (K_i = 6.0 nM). Other compounds related to **3** such as *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-homopiperidinyl)ethylamine (**19**) exhibited K_i = 0.17 nM ([^{3}H]-(+)-3-PPP). The determinants for high σ receptor affinity of **3** were examined by manipulation of this structure in a number of different ways. The high efficacy of these compounds for the σ receptor, their relative chemical simplicity and ease of synthesis, and their high degree of selectivity identifies *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (**3**) and related compounds as a highly promising base for determination of the functional role of σ receptors as well as the development of novel therapeutic agents.

Introduction

σ receptors have attracted much attention due to their ability to bind with significant affinity a number of psychoactive compounds or compounds with other activities (see ref 1 for a review). Among these are haloperidol and other typical neuroleptics,[2,3] the dissociative anesthetic phencyclidine,[4] the antitussive dextromethorphan,[5] and the steroid hormone progesterone.[6] Though many σ ligands bind to other receptors, σ sites are distinct from any known neurotransmitter or hormone receptor. Attempts to define a functional role(s) for σ sites have resulted in its implication in several physiological and biochemical processes. Among these are (1) regulation of motor behavior and postural tone,[7-9] (2) negative modulation of the phosphoinositide response to muscarinic cholinergic agonists,[10-12] (3) regulation of smooth muscle contraction,[13-15] and (4) neuroprotective activity.[16] The ability of σ ligands to affect motor systems and protect from neuronal damage suggests that selective σ compounds may be useful therapeutic agents in the treatment of motor disorders such as dystonia[17] and protection from the

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