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BIOLOGICAL PROPERTIES OF (R)-4-BENZAMIDO-5-OXOPENTANOIC BASIC DERIVATIVES AS CCK-ANTAGONISTS

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Abstract: The biological activities of a new class of (R)-4-benzamido-5-oxopentanoic basic derivatives as CCK-antagonists are described, and compared to that of the acidic parent compounds such as lorglumide,loxiglumide and CR 1795.

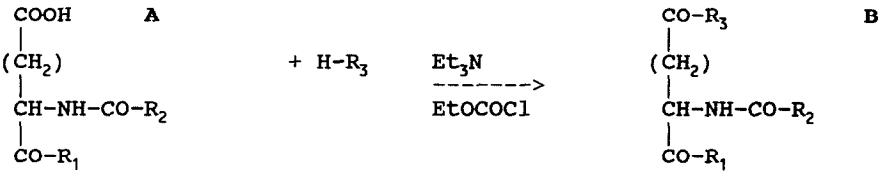
The gastrointestinal polypeptide hormones gastrin and cholecystokinin (CCK) are closely related chemically. Both have a common terminal pentapeptide sequence, but they exhibit different biological effects on their target tissues.[1,2] CCK is also widely distributed throughout in the brain and it has been hypothesized that it may function as a neuromotransmitter in the CNS.[3] 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).[4] A third receptor subtype, which appears to be closely related to the CCK-B type, is the stomach gastrin receptor. We have arbitrarily termed the central CCK receptor as CCK-B_2 and the peripheral gastrin receptor as CCK-B_1, respectively.[5] In the recent years many attempts have been made to discover potent and specific antagonists of both the CCK-A and CCK-B receptor subtypes.[6]

Among the amino acid derivatives CR-1409 (lorglumide),[7] CR-1505 (loxiglumide)[8] and CR-1795,[5] i.e. (R)-4-(2-naphthoylamino)-5-(dipentylamino)-5-oxopentanoic acid, are the most potent and specific CCK-A antagonists, with low affinity with the CCK-B receptor.

Appropriate chemical manipulations of the structure of lorglumide led to new molecular entities exhibiting potent and selective antagonistic activities on CCK-B and gastrin receptors. Compound CR-2194, i.e. (R)-4-(3,5-dichlorobenzamido)-5-(8-azaspiro[4.5]decan-8-yl)-5-oxopentanoic acid is the optimized CCK-B/gastrin antagonist in this series.[5]

The aim of this investigation was to investigate if CCK-A and CCK-B antagonism of 4-benzamido-5-oxopentanoic acid derivatives depends not only on the steric hindrance of both their benzamido and alkylamido moieties, but also on their acidic character. Therefore compounds CR-2194, its homologous CR-2325, i.e. (R)-4-(3,5-dichlorobenzamido)-5-(3-azaspiro[5.5]undecan-3-yl]-5-oxopentanoic acid, CR-2093,[5] i.e. (R)-4-(3-chlorobenzamido)-5-[(3,3-

dimethylbutyl)-amino]-5-oxopentanoic acid, CR-2227 (compound 52),[5] i.e. (R)-4-(3,5-dichlorobenzamido)-5-[2-(1-adamantyl)-ethylamino]-5-oxopentanoic acid, (R)lorglumide and CR-1795 (compounds A) were condensed with the appropriate amine ($H-R_3$) to obtain basic derivatives (compounds B), according to the scheme 1:



Scheme 1.

The synthesis of CR-2345 (compound 1, Table 1), was achieved from CR-2194 as follows: CR-2194 was reacted in THF at -10°C with triethylamine and ethyl chloroformate for 20 min. N-methyl-piperazine was added dropwise at the same temperature and the reaction continued for 1 h at -10°C and for 3 h at room temperature. The evaporation of the solvent under reduced pressure left a residue, which was washed with water to remove the excess unreacted amine. The crude product was dissolved in acetone and treated with an acetonic solution of HCl. The precipitated CR-2345 (HCl) was filtered and recrystallized from ethyl acetate/ethyl ether 3:1.Yield 75%.

The compound exhibited satisfactory IR, ^1H NMR and elemental analysis ($C_{26}H_{37}Cl_3N_4O_3$). Optical purity by HPLC, higher than 99%.$[\alpha]_D^{25}$= -66.4° ($CHCl_3$). mp (uncorrected): 166-168°C.

The physicochemical characteristics of the new (R)-4-benzamido-5-oxopentanoic basic derivatives, synthesized with this procedure, are given in Table 1.

Biological results and discussion

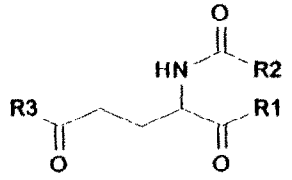
The results obtained from CCK-A and B_2 binding and gastrin (CCK-B_1) antagonism are presented in Table 2. The introduction of basic groups in the structure of the CCK-A and CCK-B antagonists, brought about different results.

Among the CCK-B antagonists CR-2194 and CR-2325, the best substitution was obtained when R_3 was the N-Me-piperazinyl group (compounds 1, coded CR-2345, and 6), that retain the antagonistic activity on gastrin receptors comparable with the acidic parent compounds. In fact, CR-2345 seems to be even more effective than CR-2194 in both in vitro (CCK-B_2) and in vivo (CCK-B_1) models of CCK-B/gastrin antagonism for example , whereas its anti-CCK-A activity is weak.

The anti-gastrin activity of CR-2345 is stereospecific, because its (S)-enantiomer, compound 11, is about 100 times less effective *in vitro* and it is devoid of any activity *in vivo*.

The introduction in R_3 of branched aliphatic tertiary amino groups (compounds 3 and 4), as well as the introduction of the dibasic piperazino group (compound 5), produced a complete loss of antigastrinic activity *in vivo*.

Table 1: Physical Properties of (R)-4-Benzamido-5-oxopentanoic Basic Derivatives (1-11) Prepared by Scheme I.



Comp.	R1	R2	R3	Formula[a]	M.p. °C	$[\alpha]_D$[b]
1	8-Azaspiro[4.5]decan-8-yl	3,5-$Cl_2C_6H_3$	4-Methylpiperazin-1-yl	$C_{26}H_{37}Cl_3N_4O_3$	168	-66.4
2	8-Azaspiro[4.5]decan-8-yl	3,5-$Cl_2C_6H_3$	4-(Ethylen-2-amino)morpholine	$C_{27}H_{38}Cl_2N_4O_4$[c]	72	-37[d]
3	8-Azaspiro[4.5]decan-8-yl	3,5-$Cl_2C_6H_3$	2-(Dimethylaminoethyl)amino	$C_{27}H_{38}Cl_2N_4O_7$[e]	91[f]	-18.4[g]
4	8-Azaspiro[4.5]decan-8-yl	3,5-$Cl_2C_6H_3$	2-(Diethylaminoethyl)amino	$C_{27}H_{41}Cl_3N_4O_3$	114[f]	-52.2
5	8-Azaspiro[4.5]decan-8-yl	3,5-$Cl_2C_6H_3$	4-Me-(ethylen-2-amino)piperazine	$C_{32}H_{45}Cl_2N_3O_7$[h]	166	-11.9[g]
6	3-Azaspiro[5.5]undecan-3-yl	3,5-$Cl_2C_6H_3$	4-Methylpiperazin-1-yl	$C_{27}H_{39}Cl_3N_4O_3$	132	-69.6
7	Dipentylamino	3,4-$Cl_2C_6H_3$	4-Methylpiperazin-1-yl	$C_{27}H_{43}Cl_3N_4O_3$	83[f]	-41.3
8	Dipentylamino	2-Naphthyl	4-Methylpiperazin-1-yl	$C_{31}H_{47}ClN_4O_3$	123	-30.6
9	[2-(1-Adamantyl)ethyl]amino	3-ClC_6H_4	4-(Ethylen-2-amino)morpholine	$C_{30}H_{43}ClN_4O_4$[c]	189	+11.5
10	3,3-Dimethylbutylamino	3-ClC_6H_4	4-Methylpiperazin-1-yl	$C_{23}H_{36}Cl_2N_4O_3$	115[f]	-37.3
11	8-Azaspiro[4.5]decan-8-yl	3,5-$Cl_2C_6H_3$	4-Methylpiperazin-1-yl	$C_{26}H_{37}Cl_3N_4O_3$	166	+66.5[i]

[a]: hydrochloride, [b]: c=2 in chloroform; [c]: free base; [d]: c=4 in methanol; [e]: oxalate; [f] amorphous; [g]: c=1 in methanol; [h]: maleate; [i]: (S)-isomer of compound 1.

With regard to the affinity with the peripheral CCK-A receptor (binding to the rat pancreatic acini), the introduction of the basic N-Me-piperazinyl group in the structure of the CCK-A antagonists CR-1795 and R-lorglumide, gave compounds 7 and 8 which exhibited reduced CCK-A antagonism. Their affinity at the CCK-A receptor is in fact about 50 times lower than that shown by CR-1795 and R-lorglumide, the acidic parent compounds. Both compounds 7 and 8 are devoid of any anti-gastrinic activity *in vivo*. Furthermore, compounds 9 and 10, which both carry a secondary amido group, in α position, retain the antigastrin activity *in vivo* exhibited by their acidic parent compounds, as well as being practically devoid of any anti-CCK-A and anti-CCK-B_2 activity. These results seem to confirm that the stomach gastrin receptor (CCK-B_1) is different from the central CCK receptor (CCK-B_2).[5]

Table 2: Comparison of biological activities of acidic and basic 4-benzamido-5-oxopentanoic derivatives.

Compound	IC_{50}^{a} μM		ID_{50}^{b} (B_1)
	CCK-A	CCK-B(B_2)	mg/kg
1 (CR-2345)	6.6 (4-11)	0.7 (0.5-1)	9.0 (6-13)
2	13.9 (6-35)	2.8 (2-5)	12.8 (9-18)
3	31.3 (16-61)	12.5 (6-27)	IN[c]
4	18.5 (8-41)	2.6 (1-7)	IN[c]
5	12.8 (10-17)	3.3 (3-4)	IN[c]
6	5.0 (3-8)	0.6 (0.4-1)	8.5 (6-11)
7	1.8 (1-3)	50.0 (19-135)	IN[c]
8	1.5 (1-2)	16.0 (12-21)	IN[c]
9	IN(>100)	42.2 (31-57)	19.0 (13-28)
10	48.8 (27-88)	103.8 (61-176)	21.2 (17-27)
11	35.7 (15-86)	120.5 (80-181)	IN[c]
(R)-lorglumide	0.05 (0.03-0.1)[D]	5.6 (4-8)	IN[c]
CR-1795	0.03 (0.02-0.05)[D]	3.8 (3-5)	IN[c]
CR-2194	13.5 (10-18)[D]	1.4 (1-2)	11.0 (8-15)[D]

[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 25 pM [^{3}H] [N-methyl-N-leucine]CCK-8 in guinea pig brain cortex (CCK-B or B_2). [b]ID_{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 ug/kg per h of pentagastrin I.V. infusion. [c]IN=the antisecretive effect of 30mg/kg is less then 20%. [D]Values drawn from cited literature[5].

Recently several potent and specific non- peptide CCK-B antagonists have been discovered, for example, the benzodiazepine L-365,260[9] and the α-methyltryptophan derivative CI-988.[10] Their potential therapeutic uses include treatment of gastro-intestinal disorders, possibly linked to antagonism of the peripheral CCK-B_1 receptor and, interestingly, treatment of anxiety disorders (through the interaction with the central CCK-B_2 receptor).[11]

Testing of these (R)-4-benzamido-5-oxopentanoic basic derivatives as potential anxiolytics is presently in progress. Preliminary results obtained with CR-2345 in the mouse black-white test box and on elevated plus-maze with the rat over the range 0.1-10 mg/kg IP show an anxiolytic activity for the compound, higher than that exhibited at the same doses by CR-2194, the parent acid compound, and comparable to diazepam dosed at 1 mg/kg IP.

In the black-white test box 0.1 mg/kg of CR-2345, given IP 20 min before the 5 min test period, increased significantly (25%; $p < 0.05$; n=50) the time spent on the illuminated side of the box. Similarly, on elevated plus-maze with the rat, CR-2345, at 1 mg/kg IP 30 min before test, augmented significantly (33%; $p < 0.05$; n=20) the percentage time spent on the open arms.

Biological Assays:

CCK-A receptor binding assays were carried out on rat pancreas by the collagenase method previously described.[12]

CCK-B_2 receptor binding assays were carried out on membranes of guinea-pig cerebral cortex as previously described[13] with modifications. Tissue (about 1.5 mg) was homogenized in ice-cold 10 mM Hepes (pH 7.4) and centrifuged twice at 4°C for 15 min at 48,000xg. The final membrane pellet was suspended in tissue buffer (10 mM Hepes, 118 mM NaCl, 4.7 mM KCl, 5.0 mM $MgCl_2$, 1.0 mM EGTA, pH 7.4) to a concentration of 100 mg/ml. Protein concentration was determined according to the method of Bradford[(14)]. Cortical membranes (0.35-0.4 mg of protein/tube), (^{3}H)(N-Me,Nle^{28,31}) CCK-8 (50000 dpm/tube) and displacing agents were incubated for 150 min at 25°C. All the components, other than cortical membranes suspension, were prepared in an assay buffer consisting of 1 mg/ml of BSA, 50 μM bestatin and 0.1 mg/ml of bacitracin dissolved in tissue buffer. Bound radioligand was separated by rapid filtration on glass fiber filters GFB (Whatman), pre-treated for at least 1 h with 0.1% BSA solution; the filters were washed three times with 4.0 ml of ice-cold normal saline and counted in a liquid scintillator.

In vivo antisecretory activity in rats (CCK-B_1 antagonism) was performed in the perfused rat stomach according to the method of Ghosh and Schild,[15] with slight modifications.[5] Anxiolytic activity in rats was performed on elevated plus-maze and in the mouse black-white test box according to the methods of Costall et al.[16,17]

References and Notes

1. Stening, G.F.; Grossman, M.I. Am. J.Physiol. **1969**, 217, 262-266.
2. Yorpes, J.E.; Mutt, V. Secretion, Cholecystokinin, Pancreozimine and Gastrin; Springer Verlag; New York, 1973;pp 1-79.
3. Dockray, G.J.; Hutchinson, R.A.; Harris, J.B.; Gregory R.A.; Runswick,M.J. Nature **1978**, 274, 711-713.
4. Steigerwalt, R.W.; Williams, J.A. Regul. Pept. **1984**, 8, 51-59.

5. Makovec, F.; Peris, W.; Revel, L.; Giovannetti, R.; Mennuni, L.; Rovati, L.C. J.Med.Chem. **1992**, 35, 28-38.
6. Evans, B.E. Drugs of the Future **1989**, 14, 971.
7. Makovec, F.; Chistè, R.; Bani, M.; Revel, L.; Setnikar,I.; Rovati, A.L. Eur.J.Med.Chem. **1986**, 21, 9-20.
8. Setnikar, I.; Bani, M.; Cereda, R.; Chistè, R.; Makovec, F.; Pacini, M.A.; Revel, L.; Arzeinm.Forsch./Drug Res. **1987**, 37, 1168-1171.
9. Lotti, V.J.; Chang, R.S.L. Eur.J.Pharm. **1989**, 162, 273-280.
10. Horwell, D.C.; Hughes, J.; Hunter, J.C.; Pritchard, M.C.; Richardson, R.S.; Roberts. E.; Woodruff, G.N. J.Med.Chem. **1991**, 34, 404-414.
11. Woodruff, G.N.; Hughes, J.Annu.Rev.Pharmacol.Toxicol. **1991**, 31, 469.
12. Innis, R.B.; Snyder, S.H. Proc. Natl.Acad.Sci.U.S.A. **1980**, 77, 6917-6921.
13. Knapp, R.J.; Vaughn, L.K.; Fang, S.N.; Bogert, C.L.; Yamamura, M.S.; Hruby, V.J.; Yamamura, H.I. J.Pharmacol.Exp.Ther. **1990**, 255,1278-1286.
14. Bradford, M.M.; Anal.Biochem. **1976**, 72, 248-254.
15. Ghosh, N.M.; Schild, H.O. Br.J.Pharmacol. **1958**, 13, 54-61.
16. Costall, B.; Kelly, M.E.; Tomkins, D.M. Br.J.Pharmacol. **1989**, 96, 312P.
17. Costall, B.; Jones, B.J.; Kelly, M.E.; Naylor, R.J.; Tomkins, D.M. Pharmacol.Biochem.Behav. **1989**, 32, 777-785.