Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 2, pp. 169-174, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(96)00609-9

DUAL CCK-A AND -B RECEPTOR ANTAGONISTS (I). C9-METHYL-1,4-BENZODIAZEPINES

Seiichiro Tabuchi,[a] Harunobu Ito,[a] Hajime Sogabe,[a] Masako Kuno,[a] Ikuyo Katsumi,[b] Naoko Yamamoto[b], Hitoshi Mitsui[b] and Yoshinari Satoh[a]*

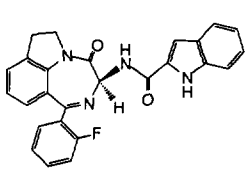
a) *New Drug Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6, Kashima, Yodogawa-ku, Osaka 532, Japan.*

b) *Materials And Functions Research Laboratories, Nippon Shokubai Co., Ltd., 5-8, Nishi Otabi-cho, Suita 564, Japan.*

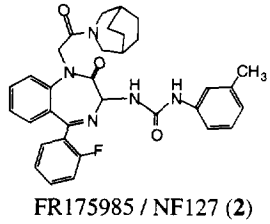
Abstract : A novel series of potent CCK-A and CCK-B dual antagonists has been prepared which incorporate a methyl substituent at the 9 position of a 1,4-benzodiazepine ring system. FR193108 ((+)-**11**) was selected for further biological evaluation, and is expected to be more efficacious than CCK-A selective antagonists for the treatment of pancreatitis, since it has high and well-balanced affinities for both CCK-A and -B receptors.

Introduction

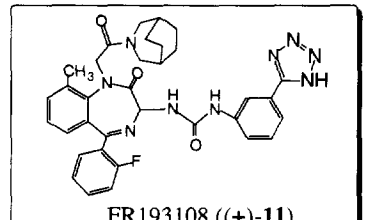
Cholecystokinin (CCK), a gastrointestinal peptide hormone plays an important role in regulating various target organs such as pancreas, gallbladder and gut. CCK is also one of the most widely distributed neuropeptides in the central nervous system (CNS) and acts as a putative neuromodulator or neurotransmitter.[1] CCK receptors have been divided into two sub-types, CCK-A and CCK-B by using various kinds of antagonists and agonistic CCK peptide fragments.[2,3,4] We have already described a novel and potent CCK-A selective antagonist, FK480 (**1**).[5] In our subsequent research for a CCK-B selective antagonist, leading to FR175985 / NF127 (**2**),[6] we discovered several compounds which had antagonistic properties against both CCK-A and CCK-B receptors, although their activities were not so potent.



FK480 (**1**)



FR175985 / NF127 (**2**)



FR193108 ((+)-**11**)

We postulated that a dual antagonist of the CCK-A and -B receptors may be more efficacious for the treatment of pancreatitis than the CCK-A selective receptor antagonists based on the following reasoning. Firstly, it is well known that the lowering of pH in the duodenum by gastric acid is one of the important factors in acceler-

ating pancreatic juice secretion, which is considered to be an exacerbating factor of pancreatitis.[7] For that reason, inhibitors of gastric acid secretion such as histamine H_2 receptor antagonists and proton pump inhibitors are often prescribed for the treatment of pancreatitis, and in this connection the CCK-B/gastrin antagonists inhibit gastric acid secretion. Secondly, although it is known that CCK-A antagonists inhibits pancreatic exocrine secretion on the one hand, they stimulate gastric acid secretion as followings. It has been recently reported[8] that the gastric acid secretion in response to food intake was accelerated by loxiglumide, a CCK-A receptor antagonist. In addition, FK480 (**1**) was shown to increase gastric acid secretion stimulated by intravenous CCK-8 (unpublished data), suggesting that CCK-A receptors are inhibitory in the gastric acid secretory process. It is thus considered that a CCK-A and -B dual antagonist could suppress both pancreatic juice and gastric acid secretion, and may be expected to be more efficacious than CCK-A selective receptor antagonists in the treatment of pancreatitis. Furthermore, when CCK-A selective and CCK-B selective antagonists were jointly administered, pancreatic exocrine secretion was inhibited more profoundly than when treated separately.[9]

This paper describes the first success[10] in discovering potent CCK-A and -B dual antagonists from a novel series of 1,4-benzodiazepine derivatives possessing a methyl substituent at the 9 position of the ring system.

Chemistry

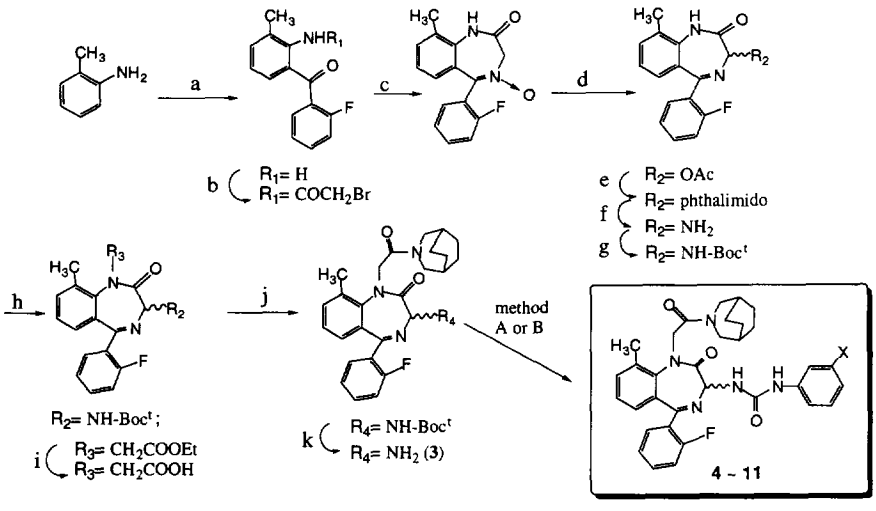
By comparison of the stereo structures of **1** and **2**, we postulated that introduction of a methyl group at the 9 position of **2** would introduce steric repulsion with the N1 side chain and the resultant total stereo structure would become close to that of **1**, leading to the possibility of dual inhibition.

3-Amino-1-(3-azabicyclo[3,2,2]nonan-3-yl)carbonylmethyl-2,3-dihydro-5-(2-fluorophenyl)-9-methyl-2-oxo-1H-1,4-benzodiazepine (**3**), the key intermediate of this series, was obtained as shown in Scheme 1. Regioselective Sugasawa reaction[11] of *o*-toluidine with 2-fluorobenzonitrile gave 2-amino-3-methyl-2'-fluorobenzophenone, which was bromoacetylated and followed by treatment with hydroxylamine to afford 9-methyl-5-(2-fluorophenyl)-1,4-benzodiazepine 4N-oxide. This oxide was rearranged with acetic anhydride to the 3-acetoxy-1,4-benzodiazepine derivative, which was substituted with a phthalimide moiety in the presence of a large excess of sodium iodide in dimethylformamide. The phthalimide was converted to the amine by treatment with hydrazine hydrate and was followed by protection with a *t*-butoxycarbonyl group. N1 alkylation with ethyl bromoacetate, alkali hydrolysis of the ester and amidation of the resulting carboxylic acid with 3-azabicyclo[3,2,2]nonane in the presence of 1-hydroxybenzotriazole (HOBT) and *N*-dimethylaminopropyl-*N'*-ethylcarbodiimide (WSCD), followed by deprotection of the *t*-Boc group with 4*N*-hydrogen chloride in ethyl acetate gave the intermediate amine (**3**). The target compounds (**4** ~ **11**) were synthesized from **3** by two methods: (i) reaction with isocyanates (method A), (ii) reaction with 4-nitrophenyl carbamate derivatives (method B).

It was found in ^{1}H-NMR spectra that the chemical shift differences of the doublet AB two protons of the methylene in the N1 side chain were all about 1.0 ppm in compounds **4**~**11**, compared to that of compound **2**, which was 0.30 ppm. This suggests that the steric repulsion between the C9 methyl and methylene of the N1

side chain in compounds **4~11** is larger than that of the C9 proton and N1 side chain in compound **2**, and it was expected that this repulsion induces a conformational change in the seven membered diazepine ring to become fairly similar to that of compound **1**.

Scheme 1



Reagent : (a) 2-F-C_6H_4-CN, BCl_3, $AlCl_3$; 2*N*-HCl, (b) $BrCH_2COBr$, Py, (c) NH_2OH•HCl, NaOH, (d) Ac_2O, (e) Phthalimide-K, NaI, (f) NH_2NH_2•H_2O, (g) *t*-Boc_2O, Et_3N, cat. NH_2OH•HCl, (h) NaH, $BrCH_2COOEt$, (i) 1*N*-NaOH, (j) 3-azabicyclo[3,2,2]nonane, HOBT, WSCD•HCl, Et_3N, (k) 4*N*-HCl in EtOAc.

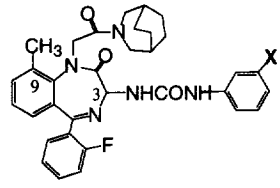
Biological Results and Discussion

Biological evaluations were performed by means of the following tests;[12] (i) inhibition of ^{125}I-CCK-8 binding to the guinea-pig cerebral cortex, (ii) inhibition of ^{125}I-CCK-8 binding to the rat pancreas and (iii) the ratio of the respective IC_{50} values. The results are summarized in Table I in comparison with the reference devazepide (MK329) and FK480 (**1**) for CCK-A selective antagonists, and L-365,260 and FR175985/NF127 (**2**) for CCK-B selective antagonists, respectively.

Initially, **4** (X=CH_3) was prepared in order to compare with **2**. The affinity for CCK-B receptors was found to be decreased by 5-fold, whereas that for CCK-A receptors increased by about 36-fold when compared with **2**. This means that **4** possesses CCK-A and -B dual antagonistic properties as we expected. Although the affinities of **5**, **6** and **7** for both CCK-A and CCK-B receptors were found to be fairly close, their affinities for the CCK-B receptor were found not to be enough for further evaluation when compared with **4**. **8** and **9** were as potent as compound **4**, but had poor water-solubilities, precluding *iv* administration. According to the literature report[13] that introduction of acidic moieties as substituent X were effective in increasing the affinity for the CCK-B receptor, we prepared **10** (X=COOH) and its bioisostere **11** (X=tetrazole) with the concurrent expectation of good solubilities in alkaline water. **10** was found to have lower activity in comparison with **4**,

but **11** displayed slightly decreased CCK-B and slightly increased CCK-A receptor binding activities respectively when compared with **4**, and the ratio (A / B) was smaller than that of **4**.

Table I



No.	X	C3 stereo ($[\alpha]_D$)	synthetic method	IC_{50} (nM)[a] for CCK-B	IC_{50} (nM)[b] for CCK-A	selectivity[c] A/B
MK329[d]				245	0.08	0.00033
FK480 (1)[e]		*S* (>99.5% ee)		310	0.67	0.0021
L-365,260[d]				2.0	280	140
FR175985/ NF127 (2)		+33.7° (c 0.5 $CHCl_3$)[f] (>99.5% ee)		0.087	62	710
4	CH_3	*RS*	A	0.47	17	36
5	Cl	*RS*	A	2.0	7.4	3.7
6	Br	*RS*	A	1.8	5.1	2.8
7	OCH_3	*RS*	A	1.0	7.3	7.3
8	SCH_3	*RS*	A	0.37	21	57
9	$COCH_3$	*RS*	A	0.84	13	15
10	COOH	*RS*	B	0.96	160	170
11		*RS*	B	0.68	14	21
(-)-**11**		-97.2° (c 0.5 $CHCl_3$)[f] (97.6% ee)	B	6.4	670	110
(+)-**11** (**FR193108**)		+82.7° (c 0.5 $CHCl_3$)[f] (85.6% ee)	B	0.38	9.2	24

a) Inhibition of ^{125}I-CCK-8 binding to guinea-pig cerebral cortical membranes, b) Inhibition of ^{125}I-CCK-8 binding to rat pancreatic membranes, c) Ratio of IC_{50} values obtained by CCK-A and CCK-B receptor binding assays, d) see reference 15, e) see reference 5, f) The absolute configuration has not been determined.

Optical Resolution of Compound 11

In order to confirm that the potent activities of **11** to the respective receptors was not due to enantiomers, since it is claimed that in general in the 1,4-benzodiazepine ring system the CCK-A selective antagonists are *S*-isomers and the CCK-B selective antagonists are *R*-isomers, we prepared the respective enantiomers *via* an

optical resolution of the intermediate **3** according to a similar method already reported.[14]

Thus, the intermediate racemic amine (**3**) was acylated with *N*-*t*-butoxycarbonyl-*L*-phenylalanine in the presence of WSCD and HOBT followed by deprotection with 4*N*-hydrogen chloride in ethyl acetate to afford a diastereo mixture, which could be separated by high pressure liquid cromatography (HPLC) eluting with a mixture of chloroform and methanol (100 : 1) to afford (3*S*)-3-(*S*-phenylalanyl)amino- and (3*R*)-3-(*S*-phenylalanyl)amino-1,4-benzodiazepine derivatives respectively. Edman degradations were performed on the respective separated diastereoisomers obtained above to give the optically active 3-amino intermadiates, which were then converted to the respective optically active target ureas ((-)-**11** and (+)-**11**) by treatment with 4-nitrophenyl 3-(tetrazol-5-yl)phenylcarbamate and triethylamine in DMF at ambient temperature.

Although the absolute configurations of (-)-**11** and (+)-**11** should be determined as being the same as those of the precursor amines, since configurational inversion does not occur in the urea formation reaction, the absolute configuration of the optically active intermediate amines has not been determined yet despite our many trials to obtain pure crystals for X-ray crystallographical analysis of the diastereomeric salts of the amines with suitable optically active acids.

The optical purities of (-)-**11** and (+)-**11** were determined by high-pressure liquid chromatography (HPLC) with a chiral stationary-phase column (CHIRALCEL OD) to be 96.7 and 85.6 % *ee* respectively.

The (+)-isomer ((+)-**11**) was found to be more potent in both receptor binding tests than the (-)-isomer ((-)-**11**) as shown in Table I.

Conclusion

A novel series of potent CCK-A and CCK-B dual antagonists has been prepared which incorporate a methyl substituent at the 9 position of the 1,4-benzodiazepine ring system. They are expected to be more efficacious for the treatment of pancreatitis than selective CCK-A receptor antagonists, because gastric acid secretion is suppressed by their CCK-B receptor antagonistic activity concurrently with the decrease of pancreatic exocrine secretion by their CCK-A receptor antagonistic activity. Compound (+)-**11** (FR193108) was selected owing to its very high and well-balanced affinities for both CCK-A and CCK-B receptors and is now undergoing further evaluation to determine whether it inhibits pancreatic exocrine secretion more potently when compared with a CCK-A selective antagonist having the same potency in the CCK-A receptor binding assay. Additionally, development of a more practical method for optical resolution of (+)-**11** and determination of its absolute configuration are in progress.

Acknowledgements : The authors would like to acknowledge Drs. H. Tanaka, K. Sakane, Y. Motoyama and T. Yoneda for helpful discussions.

References and Notes:

1. Williams, J. A. *Biomed. Res.*, **1982**, *3*, 107.
2. Innis, R. B.; Snyder, S. H. *Proc. Natl. Acad. Sci. USA.*, **1980**, *77*, 6917.
3. Moran, T. H.; Robinson, P. H.; Goldrich, M. S.; McHugh, P. R. *Brain Res.*, **1986**, *362*, 175.

4. Hill, D. R.; Woodruff, G. N. *Brain Res.*, **1980**, *526*, 276.
5. Satoh, Y.; Matsuo, T.; Sogabe, H.; Itoh, H.; Tada, T.; Kinoshita, T.; Yoshida, K.; Takaya, T. *Chem. Pharm. Bull.*, **1994**, *42*, 2071.
6. Katsumi, I.; Satoh, Y.; Mitsui, H.; Satoh, Y.; Tabuchi, S.; Ito, H.; Sogabe, H.; Kuno, M.; Tanaka, H., *16th. Med. Chem. Symp.* (Toyama, Jap., **1996**, Nov. 27-29) *Abst.* p.155, No. 2-P-21.
7. (1) Green, G.M. *Pancreas* **1990**, *5*, 445. (2) Chen, Y. F.; Chey, W. Y.; Chang, T. M.,*Am. J. Physiol.*, **1985**, *249*, G29. (3) Guan, D.; Ohta, H.; Tawil, T., *Gastroenterology*, **1990**, *98*, 437.
8. Konturek, J. W.; Stoll, R.; Gutwiska-Konturek, M.; Konturek, S. J.; Domschke, W. *Scand. J. Gastroenterol.*, **1993**, *28*, 401. ; Burckhardt, B.; Delco, F.; Ensinck, J. W.; Meier, R.; Bauerfeind, P.; Aufderhaar, U.; Ketterer, S.; Gry, K.; Beglinger, C. *Eur. J. Clin. Invest.*, **1994**, *24*, 370.
9. FK480 (CCK-A selective antagonist / Fujisawa) and YM022 (CCK-B selective antagonist / Yamanouchi) were examined concomitantly and solely in the casein-stimulated pancreas exocrine secretion and the results will be reported in detail by our biologists in the near future.
10. "Mixed" CCK-A/CCK-B antagonists were reported by Parke Davis chemists in the course of research into PD-134308 (CI-988), a potent and selective CCK-B receptor antagonist, by inversion of the configuration of the peptoid side chain, but the possible indications of mixed CCK-A/CCK-B antagonists were not alluded to at all. See: Boden, P. R.; Higginbottom, M.; Hill, D. R., *J. Med. Chem.*, **1993**, *36*, 552.
11. Sugasawa, T.; Toyoda, T.; Adachi, M.; Sasakura, K., *J. Am. Chem. Soc.*, **1978**, *100*, 4842.
12. Ito, H.; Sogabe, H.; Nakarai, T.; Satoh, Y.; Tomoi, M.; Kadowaki, M.; Matsuo, M.; Tokoro, K.; Yoshida, K. *J. Pharmacol. Exp. Ther.*, **1994**, *268*, 571.
13. Lowe, III, J. A.; Drozda, S. E.; McLean, S.; Bryce, D. K.; Crawford, R. T.; Zorn, S.; Morrone, J.; Appleton, T. A.; Lombardo, F. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 1933. ; Chambers, M. S.; Hobbs, S. C.; Graham, M. I.; Watt, A. P.; Fletcher, S. R.; Baker, R.; Freedman, S. B.; Patel, S.; A. J. Smith, A.; Matassa, V. G. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 2303.
14. Rittle, K. E.; Evans, B. E.; Bock, M. G.; DiPardo, R. M.; Whitter, W. L.; Homnick, C. M.; Veber, D. F.; Freidinger, R. M. *Tetrahedron Lett.*, **1987**, *28*, 521.
15. Bock, M. G., *Drugs Fut.*, **1991**, *16*, 631.

(*Received in Japan* 25 *October* 1996; *accepted* 5 *December* 1996)