Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1419–1424

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel Constrained CCK-B Dipeptoid Antagonists Derived From Pipecolic Acid

Bruno Bellier, Sophie Da Nascimento, Hervé Meudal, Edith Gincel, Bernard P. Roques & Christiane Garbay * (1)

Département de Pharmacochimie Moléculaire et Structurale • INSERM U266 • CNRS URA D1500

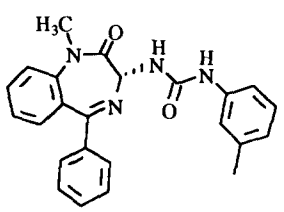
UFR des Sciences Pharmaceutiques et Biologiques • 4, avenue de l'Observatoire • 75270 PARIS Cedex 06 • FRANCE

Received 2 March 1998; accepted 28 April 1998

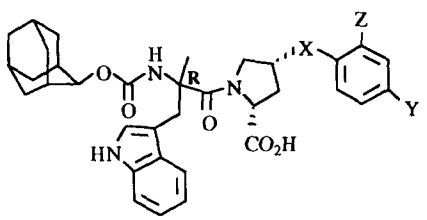
Abstract : A new series of 4-substituted pipecolic acid derivatives was prepared and incorporated into dipeptoids. The resulting products behave as moderately potent CCK-B antagonists but their constrained structure and its comparison with structurally related compounds yield valuable information about the conformational requirements for optimal recognition of the CCK-B receptor by antagonists.

Keywords : Cholecystokinin ; Peptoids ; Antagonists.

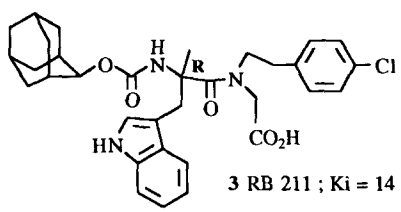
The peptide hormone cholecystokinin (CCK) is involved in a wide range of physiological effects including regulation of food intake, anxiety, cognitive processes and analgesia [1]. These actions are mediated by two receptors designated CCK-A and CCK-B, which have both been cloned and sequenced. No evidence for another structurally different CCK receptor, in terms of amino acid sequence, has been obtained to date [2].



1 L-365,260 : Ki = 15 nM ; IC_{50} = 39 nM [3]



2a X = O ; Y = Z = Cl ; Ki = 15.3 nM ; IC_{50} = 37.4 nM
2b X = O ; Y = Z = F ; Ki = 18.6 nM ; IC_{50} = 389 nM
2c X = O ; Y = NO_2, Z = H ; Ki = 32.7 nM ; IC_{50} = 507 nM
2d X = O ; Y = Z = H ; Ki = 28 nM
2e X = OCH_2 ; Y = Z = H ; Ki = 24 nM [3]

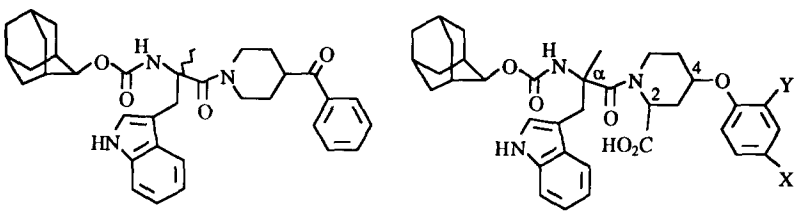


3 RB 211 ; Ki = 14 nM ; IC_{50} = 217 nM [3]

(1) ε-mail. Christiane.Garbay@pharmacie.univ-paris5.fr • Fax. (33)-1-43.26.69.18.

PII: S0960-894X(98)00231-5

Nevertheless, several experiments using CCK-B ligands have suggested the existence of two CCK-B receptor subtypes, controlling specific pharmacological actions [4-6]. Thus, the CCK-B benzodiazepine antagonist L-365,260 (**1**) was shown to bind to the CCK-B receptor following a two-site binding model [7], and we have recently demonstrated that the small, constrained molecules **2a-2c** were also able to discriminate two affinity states of this receptor [8]. These proline-based dipeptoids were designed by introducing a conformational restriction in the structure of the CCK-B specific antagonist, RB 211 (**3**) [9]. The resulting compounds were also potent and specific CCK-B antagonists, with affinities reaching values close to 10 nM, indicating that the chosen constraint forced the two substituents of the pyrrolidine ring into a good but not optimal spatial arrangement for receptor recognition.



10

Structure of the target compounds ;
α , 2, 4 indicate the chiral centres

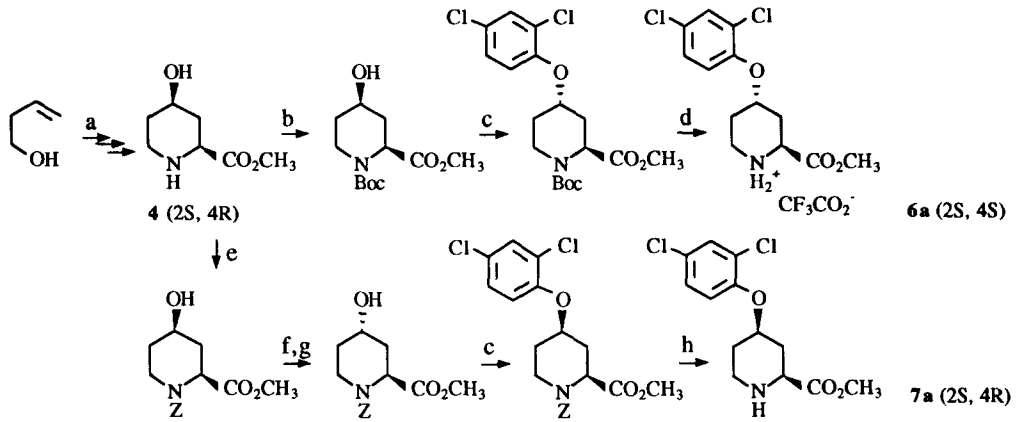
Thus, constrained derivatives of RB 211 appear to be valuable tools for the exploration of CCK-B receptor heterogeneity. Moreover, structure-affinity relationships of the proline-containing series indicated that lengthening the distance between the amide nitrogen atom and the phenyl ring was of little importance, while the position of the carboxylate could not be modified. Therefore, new restrained target compounds were designed with a piperidine ring replacing pyrrolidine in order to slightly modify the relative orientation of the aromatic moiety towards the carboxylate, without violating any of the requirements previously established in both linear and constrained series for CCK-B binding.

I • CHEMISTRY

The methodology used for the preparation of the new dipeptoids relies on the synthesis of enantiomerically pure 4-substituted pipecolic acids. A 2,4-dichlorophenoxy moiety was chosen for 4-substitution of the piperidine ring, as in compound **2a**, which discriminates two CCK-B binding sites the most clearly and has the strongest antagonist power in its series. The first key intermediates were L- and D-4-*cis*-hydroxypipecolic acid methyl esters **4** and **5** which were prepared following the method of Gillard *et al.* [10]. Mitsunobu reaction [11] of these hydroxy-aminoesters (protected as Boc) with 2,4-dichlorophenol yielded L and D-(N-Boc)-*trans*-4-(2,4-dichlorophenoxy)-pipecolic acid methyl esters **6a** and **8a** (Scheme 1).

Preparation of *cis*-4-(2,4-dichlorophenoxy)-pipecolic acid methyl esters **7a** and **9a** required a second inversion of the configuration of carbon 4, which was achieved *via* Mitsunobu esterification of formic acid with **4** or **5** and subsequent deprotection of the formyl group in strong acidic medium. This required a different protection of the amine function, this time with a Z group, which can be introduced without altering the secondary alcohol function [12] (Scheme 1).

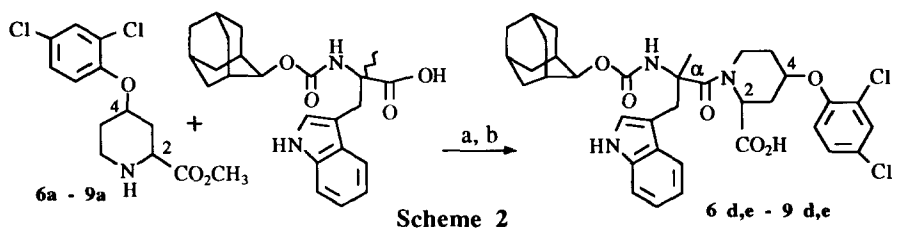
The apparently high sensitivity of the hydroxy-acids to dehydration under Mitsunobu conditions required modifications of the standard protocol in order to sufficiently increase the yield of the desired product [13]. This problem can be explained by the structure of the piperidine ring, where the trans-elimination of axial substituents is favoured, which is not the case in the proline series. This problem was not mentioned in a previous report on equivalent pipecolic acid derivatives, but in this particular case, this could be due to the protecting group used by the authors, bis-trifluoromethyl-oxazolidine [14]. In any case, conversion of the *trans* products to the *cis* ones always provided higher yields than the reverse operation.



Scheme 1

a. 1. TsCl, Et_3N ; 2. S-α-methylbenzylamine, Et_3N ; 3. Glyoxylic acid ; 4. Separation of diastereoisomers, followed by 5. H_2, $Pd(OH)_2/C$, HCl 4N in dioxane, MeOH • b. Boc_2O, NaOH 1N, dioxane • c. DEAD, PPh_3, 2,4-dichlorophenol, THF • d. TFA, CH_2Cl_2 • e. Z-Cl, $NaHCO_3$ 1N • f. DEAD, PPh_3, HCO_2H, THF • g. HCl 2N in water • h. H_2, Pd/C, MeOH. The same procedure starting from **5** [(2R, 4S) enantiomer of **4**] led to **8a** [(2R, 4R) enantiomer of **6a**] following steps b to d and **9a** [(2R, 4S) enantiomer of **7a**] following steps e to h.

Amines **6-9a** were coupled to 2-Adoc-DL-α-methyltryptophan, giving the corresponding pairs of diastereomeric dipeptoid esters **6 b,c** to **9 b,c** which were not isolated. The coupling of the two sterically hindered fragments proved difficult and needed to be performed in a minimal amount of solvent (less than 1ml/mmol of acid) with an excess (2 eq.) of amine and of coupling agent (only HATU, but not BOP, gave yields superior to 30%). Such conditions led to numerous byproducts which rendered purification difficult. Subsequent saponification led to the corresponding pairs of acids **6 d,e** to **9 d,e** (Scheme 2).



Scheme 2

a • HATU, DIEA, DMF (see text for precisions). b • NaOH 1N, dioxane, then separation of isomers. α , 2, 4 designate the chiral centres.

The *trans* isomers (trans and cis referring to the relative orientation of the piperidine ring substituants) **6d** and **6e** (**8d** and **8e**, respectively) could be separated from the mixture **6 d,e** (**8 d,e**, respectively) by column chromatography on silica gel, whereas purification of the *cis* isomers, **7d**, **7e** and **9d**, **9e**, required semi-preparative HPLC. Finally, the eight pure isomers of the compound 2-Adoc-αMeTrp-Pip[4-OPh(o,p-Cl_2)]-OH were obtained.

II • RESULTS AND DISCUSSION

1. Biological Evaluation.

All the final dipeptoids were evaluated for their capacity to inhibit [^{3}H]p CCK-8 binding to membrane preparations of CHO cells stably transfected with the rat CCK-B receptor. The ester **9c** corresponding to the acid **9e** having the highest affinity was also tested under the same conditions. The results are presented in Table 1, which also includes the characteristics of compounds **2a** and **3**, and of the closely related dipeptoid **10**, formerly described by Holladay *et al.* [15]. The affinity of the most potent CCK-B compound (**9e**), for CCK-A receptors was measured on guinea-pig pancreatic membranes [16].

Table 1

The absolute configuration at the α, C_2 and C_4 carbons is given. (c) and (t) indicate the *cis* or *trans* orientation of the piperidine ring substituents. Ki values were determined by displacement of [^{3}H]pCCK$_8$ from CHO cells (CCK-B) except for compound **11** (guinea-pig cortical membranes) and from guinea-pig pancreatic membranes (CCK-A). N.D. : not determined.

Compound	R_2	α	C_2	C_4	Ki(CCK-B, nM)	Ki(CCK-A, nM)
6d(t)	CO_2H	S	S	S	1679 ± 368	N.D.
6e(t)	CO_2H	R	S	S	791 ± 110	N.D.
7d(c)	CO_2H	S	S	R	896 ± 157	N.D.
7e(c)	CO_2H	R	S	R	543 ± 37	N.D.
8d(t)	CO_2H	S	R	R	1040 ± 84	N.D.
8e(t)	CO_2H	R	R	R	789 ± 119	N.D.
9c(c)	CO_2CH_3	R	R	S	1538 ± 59	N.D.
9d(c)	CO_2H	S	R	S	812 ± 157	N.D.
9e(c)	CO_2H	R	R	S	175.2 ± 31	3723 ± 458
2a(c)	CO_2H	R	R	R	15.3 ± 1.7	751 ± 22
3	-	R	-	-	14 ± 1	1060 ± 32
10	-	R,S	-	-	3400 ± 480	1700 ± 370

The results show a general loss of affinity, in comparison with the equivalent proline-containing dipeptoids, as exemplified by the three-fold decrease from **2a** to **9e**. It should be noted that the introduction of a supplementary methylene inverses the stereochemistry at carbon 4 while the geometry at this atom is conserved. Thus, the most favourable configuration of the piperidine ring is (2R, 4S) in **9d** and **9e**, which corresponds to the same geometry of the substituents in **2a**.

In general, compounds showing a *cis* orientation of the substituents at carbons 2 and 4 (meaning that both are located on the same side of the ring) are always more potent than their *trans* equivalents, which have affinities only in the micromolar range. As shown before for several dipeptoid series, an R configuration at the α carbon of the tryptophan residue is systematically preferred for CCK-B recognition. The critical importance of C-terminal acid can be seen by comparing **9e** and its methyl ester equivalent **9c**, or even more so with the benzoylpiperidide **10**, which bears no substituent at the alpha positions of the ring.

Indeed, the six-membered ring does not appear to force the essential features for CCK-B recognition into an optimal fit. However, the final compounds are better ligands than other similar molecules derived from CCK_4, which also organize comparable moieties (one hydrophilic and three bulky hydrophobic groups) around a six-membered skeleton [17,18], and are structurally closer to the CCK_4 tetrapeptide. This may be due to both a better choice of these groups [17] (2-Adoc instead of Boc or 1-Adoc, acid C-terminus instead of methyl ester) and a higher flexibility of the piperidine skeleton compared to oxopiperazines [18] or bicyclic oxoindolizidines [17], whose affinities are in the micromolar range.

Further biological investigation of compound **9e** showed it to be selective for CCK-B versus CCK-A receptors, which is also the case in the related proline-containing dipeptoid series [8]. Furthermore, **9e** was shown to antagonize inositol phosphate production triggered by CCK_8 in CHO cells with an IC_{50} of 356 nM, while no agonist property of this compound could be evidenced.

2. Structural and conformational analysis.

Compound **9e** was subjected to two-dimensional NMR conformational analysis using COSY, TOCSY and ROESY standard techniques at 400 MHz or 600 MHz. The particular structure of the dipeptoid studied raised questions about the conformation around the peptide bond (presence of N-*cis*/N-*trans* rotamers) and the conformation of the piperidine ring. 1D NMR spectra of **9e** and **2a** showed important similarities, especially the presence of two distinct signals for protons H_2 and H_4 of the piperidine ring, indolic NH, α-methyl, which in **2a** could only account for the occurrence of two rotamers around the proline bond. Likewise, this indicated for **9e**, that both N-*cis* and N-*trans* rotamers coexisted in a 1:3 proportion ; a strong NOE cross peak between the piperidine H_6 and Trp α-methyl signals proved the major form to be the N-*trans* one. Moreover, 2D experiments allowed the unambiguous attribution of all signals, except those of the adamantyl moiety, and strongly suggested that protons H_2 and H_4 of the ring had to be in axial positions. Thus, both substituents of the ring ought to be essentially equatorial, which is energetically more satisfying.

Computer-aided energetic minimization was performed on compounds **2a**, **2d** (data not shown), **2e** and **9e** to understand the observed loss of affinity of CCK-B the latter. Figure 1 shows the calculated structures of these compounds, after superposition of the tryptophan residues.

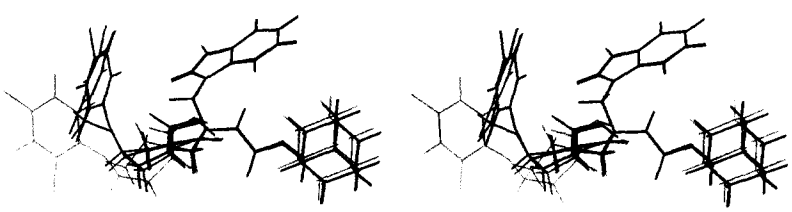


Figure 1 • Compound **2a** (blue), compound **2e** (red) and compound **9e** (green).

The two proline-containing dipeptoids (**2a** and **2e**) adopt a W-shaped conformation ; this must be due to favourable electronic interactions between the phenyl and the indole moieties. In contrast, the piperidine ring in **9e** may prevent such interactions, and favour an extended conformation. Such a difference between receptor-bound conformations of **2a** and **9e** would be sufficient to explain the difference between their affinities. Thus, this study completes former investigations which indicated that the distance between the amide nitrogen and the phenyl ring was of little importance in the proline series [8] but had to be of two carbons in the linear series, as for RB 211 [9]. Finally, the results presented here strongly suggest a W-shaped bioactive conformation of peptoid CCK-B antagonists, and this data will be useful for further investigations in other series.

ACKNOWLEDGEMENT

The authors wish to thank C. DUPUIS for the expert drafting of the manuscript and realization of figures.

REFERENCES & NOTES

1. Reviewed in Crawley, J.N. ; Corwin, R.L. *Peptides*, **1994**, *15*, 731-55.
2. Reviewed in Shulkes, A. ; Baldwin, G.S. *Clin. Exp. Pharm. Pharmacol.*, **1997**, *24*, 209-216.
3. Ki values were determined on membrane preparation of CHO cells stably transfected with the rat CCK-B receptor. IC_{50}s are the concentrations inhibiting 50% of inositol phosphate production triggered by CCK-8 (0.5 nM) in the same cells (see ref.8 for details).
4. Durieux, C. ; Coppey, M. ; Zajac, J.M. ; Roques, B.P. *Biochem. Biophys. Res. Commun.*, **1986**, *137*, 1167-1173.
5. Derrien, M. ; McCort-Tranchepain, I. ; Ducos, B. ; Roques, B.P. ; Durieux, C. *Pharmacol. Biochem. Behav.*, **1994**, *49*, 133-141.
6. Léna, I. ; Roques, B.P. ; Durieux, C. *J. Neurochem.*, **1997**, *68*, 162-168.
7. Harper, E.A. ; Roberts, S.P. ; Shankley, N.R. ; Black, J.W. *Br. J. Pharmacol.*, **1996**, *118*, 1717-1726.
8. Bellier, B. ; McCort, I. ; Ducos, B. ; Da Nascimento, S. ; Meudal, H. ; Noble, F. ; Garbay, C. ; Roques, B.P. *J. Med. Chem*, **1997**, *40*, 3947-3956.
9. Blommaert, A.G.S. ; Weng, J.H. ; Dorville, A. ; McCort, I. ; Ducos, B. ; Durieux, C. ; Roques, B.P. *J. Med. Chem*, **1993**, *36*, 2868-2877.
10. Gillard, J. ; Abraham, A. ; Anderson, P.C. ; Beaulieu, P.L. ; Bogri, T. ; Bousquet, Y. ; Grenier, L. ; Guse, I. ; Lavallée, P. *J. Org. Chem.*, **1996**, *61*, 2226-2231.
11. Mitsunobu, O. *Synthesis*, **1981**, 1-28.
12. Guttmann, S. ; Boissonnas, R.A. *Helv. Chim. Acta*, **1958**, *41*, 1852-1867.
13. *Typical experimental procedure for Mitsunobu reaction using (N-Boc)-4-hydroxypipecolic acid methyl ester :* triphenylphosphine (1.5 mmol) and diethylazodicarboxylate (1.5 mmol) were dissolved in 3 mL ice-cooled distilled THF and stirred for 1 hour. Formic acid (or respectively 2,4-dichlorophenol) (1.5 mmol) was added and the solution stirred for an additional half hour. A solution of the hydroxy acid (1 mmol in 2 mL of THF) was then added dropwise for approximately 1 hour, and the mixture was stirred overnight at r.t.. The desired compound was obtained after evaporation and column chromatography.
14. Golubev, A. ; Sewald, N. ; Burger, K. *Tetrahedron*, **1996**, *52*, 14757-14776.
15. Holladay, M.W. ; Bennett, M.J. ; Bai, H. ; Ralston, J.W. ; Kerwin, J.F.Jr. ; Stashko, M. ; Miller, T.R. ; O'Neill, A.B. ; Nadzan, A.M. ; Brioni, J. ; Lin, C.W. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 3057-3062.
16. Biological experiments were performed following the procedures described in ref.8.
17. González-Muñiz, R. ; Dóminguez, M.J. ; Martín-Martínez, M. ; Herranz, R. ; García-López, M.T. ; Barber, A. ; Ballaz, S. ; Del Río, J. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 967-972.
18. Batt, A.R. ; Kendrick, D.A. ; Mathews, E. ; Rooker, D.P. ; Ryder, H. ; Semple, G. ; Szelke, K. *Bioorg. Med. Chem. Lett.*, **1994**, *4*, 867-872.