Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 13, pp. 1421-1426, 1995
Elsevier Science Ltd
Printed in Great Britain
0960-894X/95 $9.50+0.00

0960-894X(95)00234-0

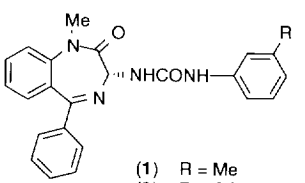
CCK_B SELECTIVE RECEPTOR LIGANDS: NOVEL 1,3,5-TRISUBSTITUTED BENZAZEPIN-2-ONES.

Monique B. van Niel,* Stephen B. Freedman, Victor G. Matassa, Smita Patel, Roy R. Pengilley and Alison J. Smith

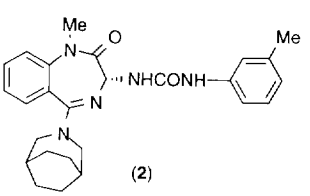
Merck, Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow , Essex, CM20 2QR, United Kingdom

Abstract: The novel 3-ureidobenzazepin-2-ones **11** and **12**, which incorporate a cationic substituent at the 5-position were designed and synthesised as ligands for the CCK_B receptor. These compounds proved to have high affinity for the CCK_B receptor and were selective over the CCK_A receptor subtype. This work further defines the role of the benzazepine template as a bioisostere for the 1,4-benzodiazepine template of earlier CCK antagonists.

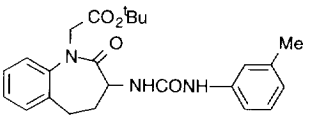
The 33 amino acid polypeptide hormone cholecystokinin (CCK) has attracted considerable interest since its presence in the central nervous system (CNS) was confirmed in 1975.[1,2] Identification of peptide and non-peptide receptor agonists and antagonists enabled the CCK receptor to be classified into two subtypes on the basis of traditional pharmaeological experiments in which receptor populations in different tissues are shown to have different rank order of potencies to these agonists and antagonists. The existence of two receptor subtypes has been confirmed by recent cloning and sequencing of genes from mammalian sources.[3] Whereas CCK_A receptors are located predominantly along the alimentary canal where they are involved in the regulation of pancreatic secretion, gall bladder contraction and gut motility, CCK_B receptors play an important role in maintaining the delicate *homeostasis* in the CNS *via* modulation of other neurotransmitters such as 5-HT, dopamine and GABA.[4] The scientific community has viewed the different distribution of receptor subtypes with interest, and it has been speculated that a CCK_B receptor antagonist could provide a new therapeutic agent for the treatment of panic and anxiety.



(**1**) R = Me
(**3**) R = COOH
(**13**) R = Br



(**2**)



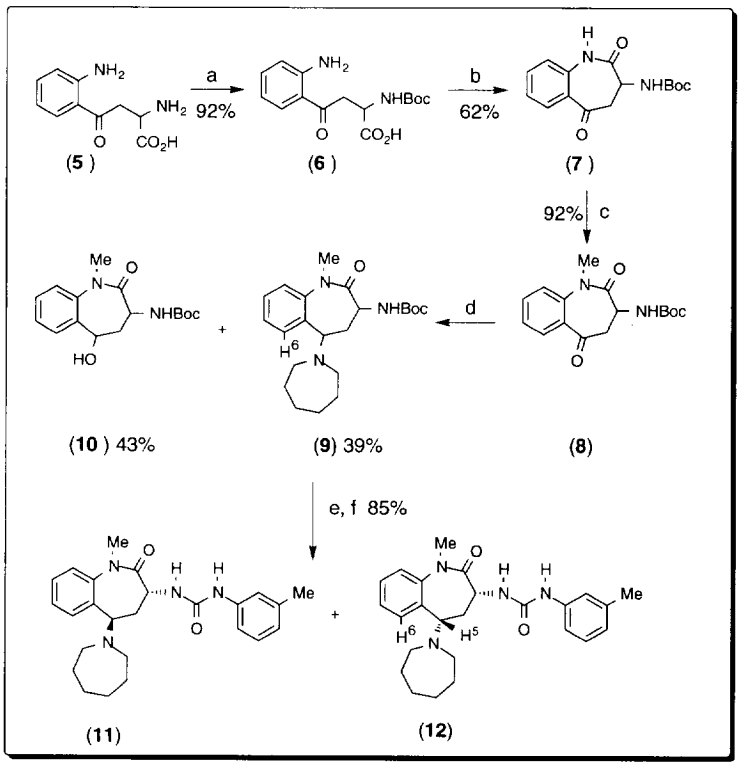
(**4**)

The development of a 1,4-benzodiazepin-2-one series of non-peptide CCK_A and CCK_B selective antagonists from the fermentation product asperlicin has been reported.[5] The urea L-365,260 (**1**) represents a landmark from those early studies due to the encouraging CCK_B receptor affinity and subtype selectivity that this non-peptide antagonist exhibited.[6] The deficiencies of L-365,260 have been discussed.[7,9] Subsequent structural refinements culminated in the identification of a water-soluble amidine [8] (e.g. **2**) and an acidic series [9] (e.g. **3**).

In parallel with extensive studies on 1,4-benzodiazepines, work in our laboratory had identified a series of 1,3-substituted benzazepines that acted as ligands at the CCK receptors.[10] Incorporation of structural motifs from the 1,4-benzodiazepine series into this novel structural class of ligands gave analogues such as **4**[10] that exhibited moderate affinity for the CCK_B receptor (**4**; IC_{50} 110nM). However, no selectivity over the CCK_A receptor was obtained. *We anticipated that the full potential of these benzazepines could be uncovered by incorporation of a substituent at the 5-position which would exploit the binding pocket at the CCK_B receptor that normally binds the substituent present at the 5-position of the 1,4-benzodiazepines.*[8,11,18]

In this communication, we disclose the results obtained from incorporation of homopiperidine at the 5-position of the benzazepine. This ring was selected since it had been shown[8] to be a high affinity group in the 1,4-benzodiazepine amidine series. It was also anticipated, by analogy with the amidine series, that incorporation of a cationic group would impart beneficial solubility characteristics compared to the C5-phenyl[11] or C5-cyclohexyl analogues, and maintain good CNS penetrability.

Scheme 1



Reagents: a) Boc_2O, Et_3N, 1,4-dioxan ; b) DCC, HOBt, THF; c) Cs_2CO_3, DMF, MeI; d) (i) $Ti(O^iPr)_4$, hexamethylenimine, THF; (ii) $NaCNBH_3$, EtOH; e) (i) HCl, EtOAc; (ii) K_2CO_3; f) *m*-tolylisocyanate, Et_3N, CH_2Cl_2.

Thus D,L-kynurenine **5** was reacted with di-*tert* - butyldicarbonate under standard conditions to give the N^{α} -*tert*-butyloxycarbonyl derivative **6** in excellent yield (Scheme 1).[12] N,N'-dicyclohexylcarbodiimide mediated cyclization gave a 62% yield of 1-benzazepine-2,5-dione **7** as the key intermediate in our synthesis.[13] Selective methylation at N-1 was achieved in high yield using iodomethane and cesium carbonate in DMF to give **8**. Attempts to introduce the secondary amine at C5 using the Borch reductive alkylation method[14] failed , presumably because of an unfavourable steric interaction between the H6 *peri* - proton (see **9**) and the methylene group of the homopiperidine ring in the iminium ion intermediate. However, reaction with an excess of titanium (IV) isopropoxide followed by sodium cyanoborohydride was successfully used to introduce the secondary amine at C5.[15] It is suggested[15] that this reaction mechanism does not involve an iminium species. The highly functionalized diastereomeric amines **9** were obtained in a 2.3 : 1 ratio. The diastereomeric alcohols **10** were the major by-product of the reaction. The diastereomeric amines **9** were not separated at this point but were carried through as a mixture into the final stages of the synthesis.

Removal of the *tert* -butyloxycarbonyl protecting group using a solution of HCl gas in ethyl acetate and reaction of the free amine with *m*-tolylisocyanate followed by separation of diastereoisomers by chromatography gave a 1:2 ratio of the novel benzazepines **11** and **12** respectively. An assignment of the relative stereochemistry was made following nOe studies. In diastereomer **12** the homopiperidine has a pseudo-axial orientation on the benzazepine ring which places H5 and the proton H6 proximal in space leading to an observed nOe between these protons. In addition, an nOe was observed between H3 and the equatorial proton on C4 and this enabled the stereochemistry for diastereomer **12** to be assigned as *cis*. The *trans* stereochemical assignment for diastereomer **11** follows from the lack of an observed nOe between the spatially remote H5 and H6 protons, and was confirmed by single crystal X-ray structural determination (Figure 1).[16]

Table 1. Binding Affinities for the C-5 Substituted Benzazepines **11** and **12.**

compound[17]	CCK_A IC_{50} (nM) [a, b]	CCK_B IC_{50} (nM) [a, c]	selectivity
11	646 (558, 748)	6.9 (6.3, 7.6)	94
12	121 (87, 169)	15.7(14.4, 17.2)	8

[a] Binding data shown as the geometric mean of three independent determinations with limits of statistical certainty shown in parentheses. [b] Binding measured in rat pancreas using inhibition of binding of [^{125} I]-CCK_8 in the radioligand assay described in reference 5a. [c] Binding measured in guinea pig cerebral cortex using inhibition of binding of [^{125} I]-CCK_8 in the radioligand binding assay described in reference 5a.

Binding of **11** and **12** to the CCK_A and CCK_B receptor subtypes was investigated using [^{125}I] CCK_8 in a radioligand binding assay to rat pancreas and guinea pig cerebral cortex respectively.[5a] **11** had high binding affinity at the CCK_B receptor and good selectivity over the CCK_A receptor (Table 1). In contrast, diastereomer **12** retained binding at the CCK_B receptor but showed only modest selectivity over the CCK_A subtype. We were very encouraged to observe that introduction of our C5 substituent had given the anticipated improvement in binding affinity when compared to the unsubstituted compound **4** (IC_{50} 110nM). The binding affinity and selectivity for racemic **11** is similar to that of the resolved compound **1** (**1**; IC_{50} 8.5nM),whilst the racemic analogue corresponding to **11** in the amidine series[8] (IC_{50} 1.25nM) shows only a marginally improved binding affinity.

Support for a correspondence between the benzodiazepine and benzazepine series comes from consideration of the conformation of **11**, as determined crystallographically[16] (Figure 1a), and overlays with the benzodiazepines **13** (Figure 1b) and **14**[18] (Figure 1c) and amidine fragment **15** (Figure 1d). In the overlays shown in Figure 1, **11** appears in black whilst the structures **13**, **14** and **15** are coloured grey.

Figure 1a.

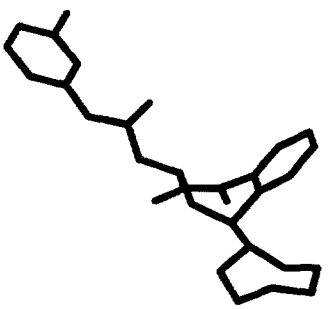


Figure 1b.

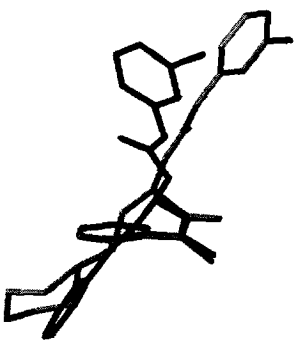


Figure 1c.

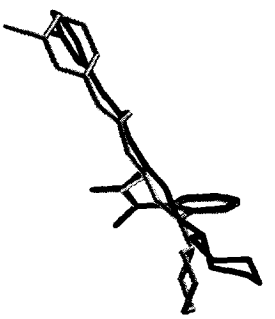
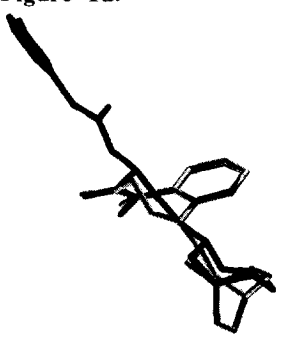
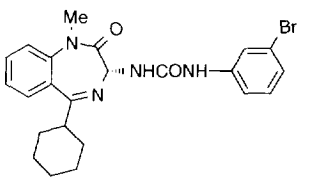
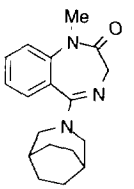


Figure 1d.





(14)



(15)

The benzazepine ring of **11** has a similar overall conformation to the CCK_B antagonists which use the 1,4-benzodiazepine ring as a template. The orientation of the homopiperidine substituent at C5 is approximately parallel to the plane of C4-C5-C6 (Figure 1a) analogous to the arrangement of the phenyl-substituted 1,4-benzodiazepine **13** (Figure 1b), but in contrast to a more perpendicular orientation for the corresponding C5 cyclohexyl ring in antagonist **14**.[18] (Figure 1c). It has been suggested[18] that the cyclohexyl ring is orthogonal in order to minimise steric interactions whereas the phenyl ring is coplanar in order to maximise conjugation with the

imine of the 1,4-benzodiazepine ring system. An overlay of **11** with the X-ray structure of the amidine fragment **15** illustrates a remarkably good fit for the C5 substituents of these molecules in the solid state (Figure 1d). Thus the homopiperidine ring of **11** occupies a similar region of space at the CCK_B receptor as the other 5-position substituents of the 1,4-benzodiazepine series of antagonists. This is obviously a contributing factor to the marked increase in binding affinity for the CCK_B receptor observed for **11** (IC_{50} 6.9nM) when compared to **4** (IC_{50} 110nM). Crystals of **12** suitable for X-ray structural determination have not been obtained. However, the nOe data discussed above suggests that the homopiperidine ring has a pseudo-axial disposition on the benzazepine ring. Inspection of Dreiding molecular models shows that there is good overlap of **12** with **11**, except for the homopiperidine ring, which by virtue of its pseudo-axial orientation, occupies a complementary region of space. The relative high affinity of **12** at the CCK_B receptor (IC_{50} 15.7nM) thus provides further support for the notion that the binding pocket at the CCK_B receptor that binds the benzodiazepine C5 substituent is extensive in accordance with our earlier findings with the C5 cyclohexyl benzodiazepine L-708,474 (**14**).[18]

This communication has described a novel series of high affinity ligands for the CCK_B receptor which incorporate a basic amine and utilise a benzazepine template as opposed to the more commonly used benzodiazepine template.

Acknowledgments.

we are grateful to Dr. Richard Herbert and Steven Thomas of the Analytical Chemistry group for obtaining nOe and mass spectral data, Dr. Richard Ball and colleagues for the X-ray structural data and Dr. Peter Hunt for providing overlays of the X-ray structural data.

References and Notes.

1. Vanderhaeghen, J-J.; Signeau, J.C.; Gepts, W. *Nature*, **1975**, *257*, 604.
2. Harro, J.; Vasar, E.; Bradwejn, J. *TiPS*, **1993**, *14*, 244.
3. (a) Wank, S.A.; Pisegna, J.R.; de Weerth, A. *Proc. Natl . Acad. Sci. USA*, **1992**, *89*, 8691.
   (b) Dethloff, L.A.; De La Iglesia, F.A. *Drug Metab. Rev.*, **1992**, *24(2)*, 267.
4. Perregaard, J.; Sanchez, C.; Arnt, J. *Curr. Opin . Ther . Pat .* January **1993**, 101.
5. (a) Chang, R.S.L.; Lotti, V.J. *Proc. Natl. Acad. Sci. USA*, **1986**, *83* , 4923.
   (b) Evans, B.E.; Bock, M.G.; Rittle, K.E.; DiPardo, R.M.; Whitter, W.L.; Veber, D.F.; Anderson, P.S.; Freidinger, R.M. *Proc. Natl . Acad. Sci. USA*, **1986**, *83*, 4918.
   (c) Evans, B.E.; Rittle, K.E.; Bock, M.G.; DiPardo, R.M.; Freidinger, R.M.; Whitter, W.L.; Lundell, G.F.; Veber, D.F.; Anderson, P.S.; Chang, R.S.L.; Lotti, V.J.; Cerino, D.J.; Chen, T.B.; Kling, P.J.; Kunkel, K.A.; Springer, J.P.; Hirshfield, J. *J.Med.Chem.*, **1988**, *31*, 2235.
   (d) Bock, M.G.; DiPardo, R.M.; Newton, R.C.; Bergman, J.M.; Veber, D.F.; Freedman, S.B.; Smith, A.J.; Chapman, K.L.; Patel, S.; Kemp, J.A.; Marshall, G.R.; Freidinger, R.M. *BioMed. Chem.* **1994**, 987.

6. (a) Bock, M.G.; DiPardo, R.M.; Evans, B.E.; Rittle, K.E.; Whitter, W.L.; Veber, D.F.; Anderson, P.S.; Freidinger, R.M. *J. Med. Chem.*, **1989**, *32*, 13.
(b) Bock, M.G. *Drugs of the Future,* **1991**, *16* (7), 631.
(c) Lotti, V.J.; Chang R.S.L. *Eur.J.Pharmacol* , **1989**, *162*, 273.

7. Chen, I.W.; Dorley, J.M.; Ramjit, H.G.; Pitzenberger, S.M.; Lin, J.H. *Drug Metab. Dispos* . **1992**, *20,* 390.

8. Showell,G.A.; Bourrain, S.; Neduvelil, J.G.; Fletcher, S.R.; Baker, R.; Watt, A.P.; Fletcher, A.E.; Freedman, S.B.; Kemp, J.A.; Marshall, G.R.; Patel, S.; Smith, A.J.; Matassa, V.G. *J.Med.Chem.*, **1994**, *37*, 719.

9. Bock, M.G; DiPardo, R.M.; Mellin, E.C.; Newton, R.C.; Veber, D.F.; Freedman, S.B.; Smith, A.J.; Patel, S.; Kemp, J.A.; Marshall, G.R.; Fletcher, A.E.; Chapman, K.L.; Anderson, P.S.; Freidinger, R.M. *J.Med.Chem.*, **1994**, *37,* 722.

10. (a) Bock, M.G; DiPardo, R.M.; Veber, D.F.; Chang, R.S.L.; Lotti, V.J.; Freedman, S.B.; Freidinger, R.M. *BioMed. Chem. Lett.*. **1993**, *3*, 871.
(b) Parsons, W.H.; Patchett, A.A.; Holloway, M.K.; Smith, G.M.; Davidson, J.L.; Lotti, V.J.;. Chang, R.S.L. *J.Med.Chem.*, **1989**, *32*, 1681.

11. After completion of our work, a publication appeared which discloses that incorporation of a phenyl group at the 5-position of **4** was important for achieving a potent and selective CCK_B receptor antagonist in this benzazepine series: Lowe III, J.A.; Hageman, D.L.; Drozda, S.E.; McLean, S.; Bryce, D.K.; Crawford, R.T.; Zorn, S.; Morrone, J.; Bordner, J. *J.Med.Chem.*, **1994** ,*37* , 3789.

12. Stewart, F.H.C.; *Aust.J. Chem.*, **1980**, *33*, 633.

13. Ball, J.B.; Wong, M.G.; Capuano, B.; Gulbis, J.M.; Mackay, M.F.; Alewood, P.F. *J. Heterocycl. Chem.*, **1990**, *27* , 279.

14. Borch, R.F.; Hassid, A.I. *J.Org.Chem.*, **1972**, *37*, 1673.

15. Mattson, R.J.; Pham, K.M; Leuck, D.J. ; Cowan, K.A. *J.Org.Chem.*, **1990**, *55*, 2552.

16. Data pertaining to the x-ray crystallographic structure of **11** has been deposited with the Cambridge Crystallographic Data Centre in accordance with the instuctions for authors.

17. All novel compounds gave satisfactory analytical data in full agreement with their proposed structure.

18. Chambers, M.S.; Hobbs, S.C.; Fletcher, S.R.; Matassa,V.G.; Mitchell, P.J.; Watt, A.P.; Baker, R.; Freedman, S.B.; Patel, S.; Smith, A.J.; *BioMed. Chem. Lett.* **1993**, *3*, 1919.

(*Received in USA* 20 *April* 1995; *accepted* 31 *May* 1995)