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L-708,474: THE C5-CYCLOHEXYL ANALOGUE OF L-365,260, A SELECTIVE HIGH AFFINITY LIGAND FOR THE CCK_B/GASTRIN RECEPTOR

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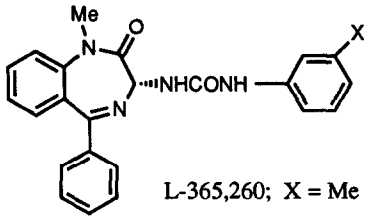
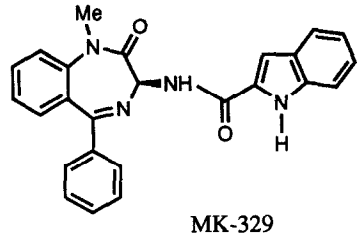
ABSTRACT: The C5-cyclohexyl analogue of the cholecystokinin type-B (CCK_B) receptor antagonist L-365,260 has been prepared. This derivative has significantly higher CCK_B affinity and markedly improved CCK_B/CCK_A receptor selectivity (6,500 v. 87-fold) than the parent compound. It is one of the most potent and selective CCK_B ligands reported to date.

The 33-amino acid polypeptide hormone cholecystokinin (CCK), and several of its molecular variants, are found in the gastrointestinal tract and in the CNS. Two distinct CCK receptor sub-types have been identified to date and these have been implicated in a number of physiological processes: the CCK_A receptor, located predominantly in the gut, is thought to play a role in pancreatic secretion, gall bladder contraction and gut motility[1,2], and the CCK_B receptor, primarily found in the brain, has been hypothesised to have a neuromodulatory role in satiety and anxiety[3]. The stomach gastrin receptor may be identical to the CCK_B receptor[4]. In an effort to clarify the physiological relevance of these receptors, extensive research effort has been invested in the discovery of selective ligands and much progress has been made, notably in the development of selective non-peptide CCK receptor antagonists[5].



L-365,260; X = Me

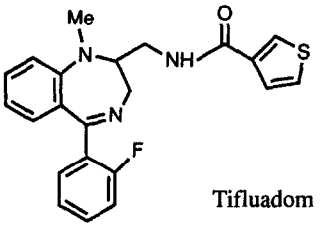
1; X = Br



MK-329

Recently, a benzodiazepine-based series of non-peptide CCK antagonists was reported which evolved from an insightful structural analysis of the fermentation product asperlicin, itself discovered by utilising receptor-based screening technology. The high affinity CCK_A-selective amide MK-329[2,6] and the CCK_B-selective urea L-365,260[7,8] and related analogues, were products of this work. Remarkably, it was discovered that, for the urea series, CCK_B or CCK_A selectivity was crucially dependent upon the stereochemistry at C3 of the

benzodiazepine ring, the (3S)-enantiomer generally being CCK_A selective and the (3R)-isomer CCK_B selective. The 5-phenyl-1,4-benzodiazepine structural motif common to these CCK antagonists is present in high affinity ligands for other receptors - for example in the opiate receptor agonist tifluadom[9] - and the suggestion has been made[6] that this motif could be considered as a "privileged" structure which facilitates binding to proteinaceous receptor surfaces, particularly perhaps those of the G-protein-linked (heptahelical[10]) superfamily. The pragmatic approach to ligand design inherent in this notion can be viewed as complementary to alternative approaches - e.g., modifying a native polypeptide[11] or modelling the three-dimensional structure of receptors and receptor/ligand complexes[12].



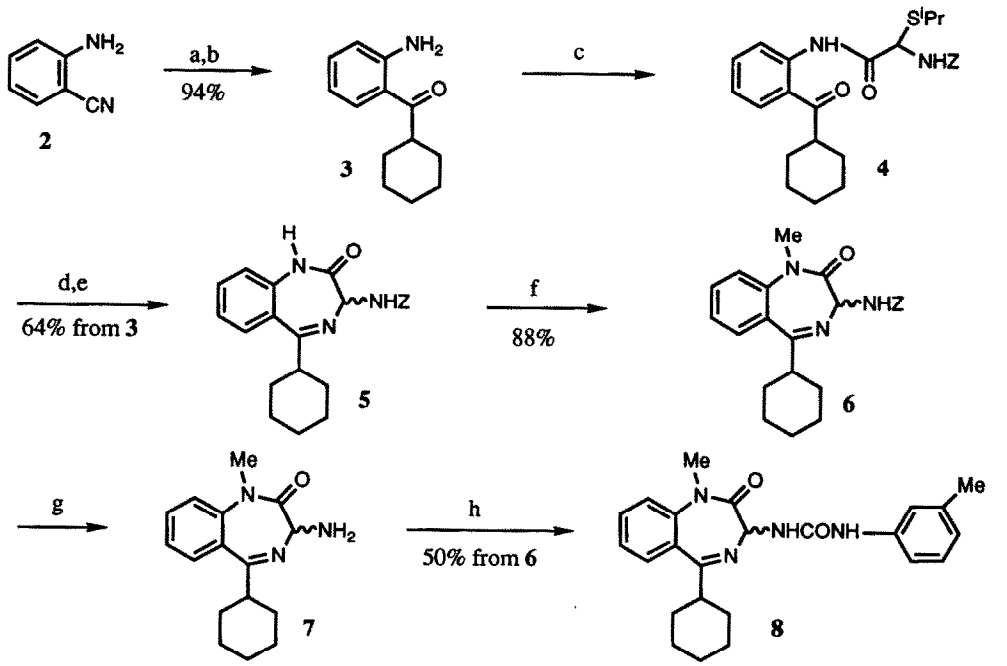
Tifluadom

Further delineation and definition of so-called privileged structures is therefore desirable and herein we report that L-708,474, the (3R)-5-cyclohexyl-1,4-benzodiazepine analogue of L-365,260, exhibits both considerably higher CCK_B receptor affinity and CCK_B/CCK_A receptor selectivity than the parent compound. In accord with earlier trends, the (S)-enantiomer is highly CCK_A-selective. A crystal structure has been obtained to allow a detailed comparison of the C5-phenyl and C5-cyclohexyl analogues.

Other than the C5-cyclohexyl analogue, the cyclopentyl and cyclobutyl analogues of L-365,260 were also prepared, to explore structure/activity relationships. The synthesis of the cyclohexyl derivative is representative of this series and is summarised in Scheme 1. Cyclohexylmagnesium chloride was reacted with 2-aminobenzonitrile (**2**) to give, after an acidic workup, the aminoketone (**3**; 94%). Transformation of (**3**) to the amide (**4**) was followed by a two-step ring-closure process, as described for the C5-phenyl analogue,[13] to afford the benzodiazepine (**5**). Methylation of the lactam NH was smoothly effected using DMF-dimethyl acetal in refluxing toluene to give (**6**) in 88% yield. Reductive cleavage of the CBZ group to give the amine (**7**), followed by condensation with m-tolyl isocyanate, afforded the urea (**8**) in 50% yield over the two steps.

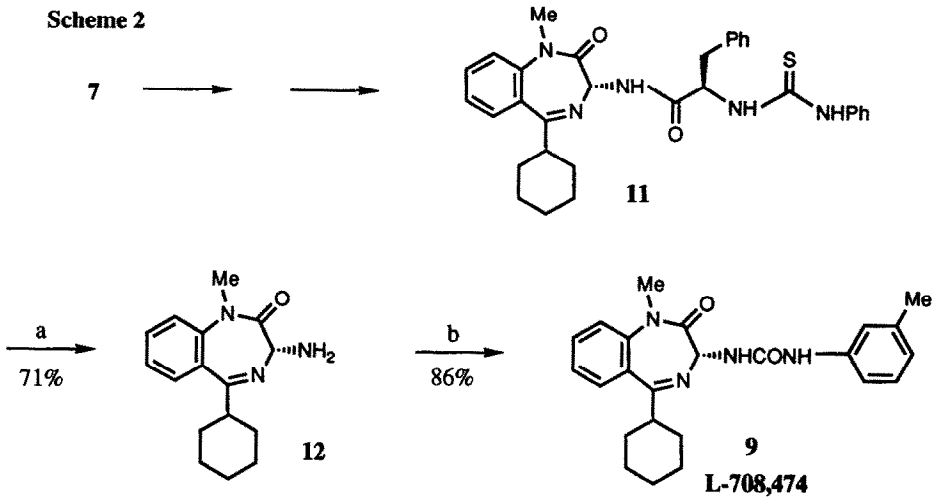
The enantiomers (**9** and **10**) of urea (**8**) were subsequently obtained using semi-preparative chiral HPLC[14]. Alternatively, the required CCK_B-selective enantiomer L-708,474 (**9**) could be prepared by an Edman degradation sequence, using similar methodology to that described for the C5-phenyl analogue[13]. The racemic amine (**7**) was converted to the (3R)-thiourea (**11**), which, on exposure to trifluoroacetic acid (ambient temperature, 30 min) afforded the (3R)-amine (**12**) in 71% yield and >99%ee. These conditions were found to be optimal and were used to prepare multigram quantities of the amine (**12**) with high optical purity. Attempts to effect the cleavage by heating (**11**) at 55°C for 15min, or even prolonged stirring at RT resulted in some racemisation (Table 1). The amine (**12**) was converted to (**9**) as described above. The CCK_B-selective enantiomer, L-708,474, was identified as the (3R)-isomer, by X-ray crystallographic analysis of the related 3-bromophenyl urea (**13**).

Scheme 1



Reagents: a) Cyclohexylmagnesium chloride, Et_2O; b) 5N HCl; c) $HO_2CCH(S^iPr)NHZ$, N-methylmorpholine, CH_2Cl_2; d) $NH_3(g)$, $HgCl_2$, THF; e) NH_4OAc, AcOH; f) DMF-dimethyl acetal, toluene, reflux; g) HCO_2H, MeOH, Pd on C; h) m-tolyl isocyanate, THF.

Scheme 2



Reagents: a) TFA, 20°C, 30 min; b) m-tolyl isocyanate, THF.

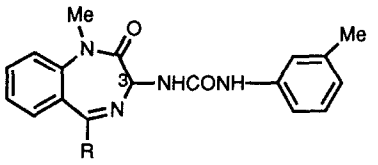
Table 1. Reaction Conditions for the Thiourea Cleavage

Time(min)	Temp.(°C)	ee[a](%)	Yield(%)
15	55	90	90
60	20	90	86
30	20	>99	70

[a] See ref.15

The binding affinities of the cycloalkyl benzodiazepines shown in Table 2 demonstrate the importance of the size of the lipophilic substituent at C5 of the benzodiazepine, and point to an advantage of cyclohexyl over phenyl for effective binding at the CCK_B receptor. The resolved cyclohexyl analogue (**9**; IC_{50} 0.28nM) was an exceptionally high affinity ligand at the CCK_B receptor and was considerably more potent than either the cyclopentyl (**14**; IC_{50} 16nM) or cyclobutyl (**15**; IC_{50} 29.9nM) analogues, the latter two being tested as racemates. L-708,474 is about thirty-fold higher in affinity than L-365,260 at the CCK_B receptor and is markedly more selective for CCK_B receptors over CCK_A (6,500-fold v. 87-fold). This data suggests that - other things being equal - the cyclohexyl ring provides a more complementary fit (*vide infra*) at the CCK_B receptor than the phenyl ring and also that the saturated ring is more discriminating against the CCK_A receptor in this enantiomeric series. As anticipated from previous work, the (3S)-enantiomer (**10**) was a selective, high affinity ligand at the CCK_A receptor (IC_{50} 0.46nM; CCK_A/CCK_B selectivity 371).

Table 2. Binding Affinities of the C5-Cycloalkyl Benzodiazepines

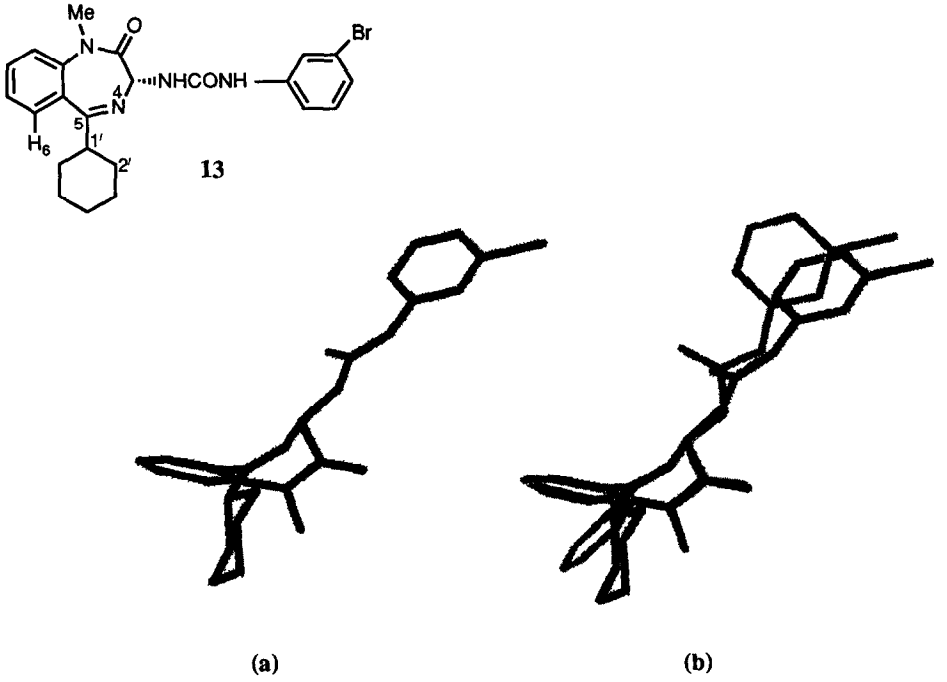


No.[b]	R	C3-Stereo.	IC_{50}(nM)[a] CCK_B[c]	CCK_A[d]
L-365,260	phenyl	R	8.50 (6.46; 11.2)	736 (585; 925)
9 (L-708,474)	cyclohexyl	R	0.28 (0.13; 0.59)	1797 (1205; 2115)
10	cyclohexyl	S	169 (91; 313)	0.455 (0.299; 0.693)
17[e]	cyclopentyl	R,S	16.0 (6.0;43)	27.1 (26;29)
18	cyclobutyl	R,S	29.9 (18.4; 48.7)	402 (340; 476)

[a] Binding results are the mean of at least four independent determinations. Statistical limits are given in parentheses. [b] All new compounds gave satisfactory analytical data in full agreement with the proposed structure. [c] CCK_B binding was measured by displacement of [^{125}I]-CCK from guinea pig cortical membranes as described in ref.2. [d] CCK_A binding was measured by displacement of [^{125}I]-CCK from rat pancreatic tissues as described in ref.2. [e] The IC_{50} value was the mean of two independent determinations.

The crystal structures of the C5-cyclohexyl derivative (**13**) and the C5-phenyl analogue[7,13](**1**) are shown in Figure 1. In the vicinity of the benzodiazepine ring, (**13**) has one major difference from the C5-phenyl derivative (**1**). The C1′-C2′ bond of the cyclohexyl ring is eclipsed with the benzodiazepine imine bond, placing the bulk of the cyclohexyl ring in a space which is approximately orthogonal to the imine, whereas the C5-phenyl ring is approximately coplanar with the imine (dihedral C2′-C1′-C5-N4 = 17°). The phenyl ring presumably enjoys conjugation with the imine whereas, clearly, the cyclohexyl ring cannot engage in such conjugative stabilisation. Additionally, in this arrangement, the cyclohexyl ring minimises steric compression with the benzodiazepine H6. Thus the cyclohexyl ring occupies a different region of space than the phenyl ring, at least in the solid state, and this may be a contributing factor to the superior receptor binding profile of (**9**). Of course the nature of the non-covalent binding interactions for the aliphatic and aromatic rings at the receptor may be inherently different. Recent site-directed mutagenesis studies on the CCK_B receptor have identified an aliphatic amino acid side-chain (Val 319 in the human receptor) which is a critical determinant of the binding affinities of non-peptide antagonists[16]. It is intriguing to speculate that the cyclohexyl ring may provide a more complementary fit than phenyl to this region of the receptor. In both molecules the conformation around the C3-N(urea) bond is such that the urea carbonyl is ca. (+) or (-) 40-45° with respect to H3. Although this leads to a different placement of the bromophenyl rings, the barrier to interconversion of these two forms might be expected to be small.

Figure 1. X-Ray Crystal Structures of (a) 13 and (b) 13 and 1



These observations have implications for the notion of privileged structures discussed above, in particular that the C5-cyclohexyl-1,4-benzodiazepine fragment should be considered alongside its phenyl counterpart . L-708,474 is one of the most potent and selective CCK_B receptor ligands yet to be reported.

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References and Notes:

1. Lotti, V.J.; Pendleton, R.G.; Gould, R.J.; Hanson, H.M.; Chang, R.S.L.; Clineschmidt, B.V. *J. . Pharmacol. Exp. Ther., 1987, 241, 103-109.*
2. Chang, R.S.L.; Lotti, V.J. *Proc. Natl. Acad. Sci. USA, 1986, 83,* 4923-4926.
3. a) Ravard, S.; Dourish, C.T. *Trends Pharmacol. Sci.*, **1990**, *11*, 271-273.
   b) For leading references to CCK functions see: Nadzam, A.M.; Kerwin Jr., J.F. *Annu. Rep. Med. Chem.*, **1991,** *26,* 191-200.
4. a) Rehfeld, J.F. *Am. J. Physiol.*, **1981**, *240,* G255-266.
   b) Beinfeld, M.C. *Neuropeptides*, **1983**, *3*, 411-427.
   c) Pisenga, J.R.; De Weerth, A.; Huppi, K.; Wank, S.A. *Biochem. Biophys. Res. Commun.*, **1992**, *189*, 296-303.
5. Bock, M.G. *Drugs Fut.*, **1991**, *16*, 631-640.
6. 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*, 4918-4922.
7. 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*, 13-16.
8. Lotti, V.J.; Chang, R.S.L. *Eur. J. Pharmacol.*, **1989**, *162*, 273-280.
9. Romer, D.; Buscher, H.H.; Hill, R.C.; Mauer, R.; Petcher, T.J.; Zeugner, H.; Benson, W.; Finner, E.; Milkowski, W.; Thies, P.W. *Nature,* **1982**, *298,* 759-760.
10. Mollon, J. *Nature* , **1991**, *351*, 696-697.
11. 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.
12. a) Hibert, M.F.; Trumpp-Kallmeyer, S.; Bruinvels, A.; Hoflack, J. *Mol. Pharmacol.* **1991**, *40*, 8-15.
    b) Strader, C.D.; Sigal, I.S.; Dixon, R.A.F. *Trends Pharmacol. Sci.* **1989** Suppl. 26-30.
13. Bock, M.G.; DiPardo, R.M.; Evans, B.E.; Rittle, K.E.; Veber, D.F.; Freidinger, R.M.; Hirshfield, J.; Springer, J.P. *J. Org. Chem.*, **1987**, *52*, 3232-3239.
14. Chiral HPLC resolution of the urea (**8**) was achieved on a dinitrobenzoylphenyl glycine column (250 x 8.0mm i.d., 5μM), using 15% ethanol in hexane as the mobile phase and a flow rate of 4ml/min.
15. The enantiomeric ratio was determined by chiral HPLC using an AGP column (100 x 4mm i.d., 5μM), with 10% acetonitrile in 10mM K_2HPO_4 (pH7) as the mobile phase and a flow rate of 0.9 ml/min.
16. Beinborn, M.; Lee, Y-M.; McBride, E.W.; Quinn, S.M.; Kopin, A.S.; *Nature*, **1993,** *362*, 348-350.