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2-NAP: A SELECTIVE, HYDROPHILIC, NON-PEPTIDE CCK_A-RECEPTOR ANTAGONIST DERIVED FROM THE CHOLECYSTOKININ C-TERMINAL DIPEPTIDE.

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Abstract. Analogues of the cholecystokinin (CCK) C-terminal dipeptide (32-33, Asp-Phe-NH_2) have been prepared and the structure-activity relationships of this series are described. The sodium salt of 2-naphthalenesulphonyl L-aspartyl (2-phenethyl)amide, (2-NAP), displayed high affinity for CCK_A receptors by its antagonism of CCK_8-stimulated guinea-pig gallbladder contraction. In addition, 2-NAP exhibits selectivity with respect to gastrin/CCK_B receptors (>300-fold) and has a low log P (-0.91, chloroform/buffer).

Cholecystokinin (CCK) was originally isolated from the porcine intestine as a 33 amino acid peptide. It shares the same C-terminal pentapeptide sequence (Gly-Trp-Met-Asp-PheNH_2) with the polypeptide hormone gastrin, which was first recognised as a potent stimulant of gastric acid secretion. Two major subtypes of CCK receptors have now been characterised, CCK_A and gastrin/CCK_B, which are found in both central and peripheral tissues[1]. The receptor types are distinguished by the relative potencies of CCK_4, which has about 1000-fold selectivity for gastrin/CCK_B receptors, and CCK_8 which is equipotent at both gastrin/CCK_B and CCK_A receptors[2].

Gastrin/CCK-receptor antagonists have been obtained from a number of sources[3], including amino-acid based compounds such as CR1505[4], CR2194[5] and A-673966; benzodiazepine ligands MK-329[7] and L365,260[8] which are respectively CCK_A and gastrin/CCK_B selective; and more recently the peptide-derived ligand PD-134308[9], which has shown selectivity for the gastrin/CCK_B receptor. The CCK_A-receptor antagonists have been shown to block CCK_8-induced gallbladder contraction and inhibition of gastric emptying[10], pancreatic secretion[11], and satiety[12] in a number of animal models. On the other hand gastrin/CCK_B-receptor antagonists have been found to be effective in a number of anxiolytic assays[13], in the potentiation of morphine analgesia[14], and peripherally in the inhibition of gastric acid secretion[15].

As part of a general strategy to design selective hormone-receptor antagonists, we have interpreted changes in the chemical structure of the natural hormone in terms of their influence on the expression of affinity and efficacy using data derived from *in-vitro* functional bioassays. This paper describes the application of this approach to the discovery of a series of selective CCK_A-receptor antagonists.

Initial structure-activity studies using small CCK fragments established that the C-terminal protected dipeptide Boc-CCK_2(32-33) **3a** retained both efficacy and affinity at gastrin/CCK_B receptors, behaving as a stimulant of

gastric acid secretion in the isolated, lumen-perfused, mouse stomach gastric acid secretion assay[16]. However, the location of the concentration-effect curve indicated that **3a** was approximately 1000-fold less potent than both Boc-CCK8 (27-33) **1** and Boc-[Leu^{31}]-CCK4 (30-33) **2**, a surrogate for tetragastrin. It was also found to be inactive in a guinea-pig gallbladder CCK_A-receptor assay [17] (Table 1.).

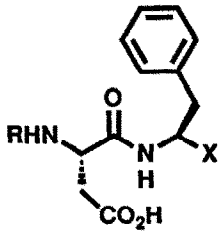
Table 1. Agonist Concentration-Effect Curve Parameters for CCK fragments[a].

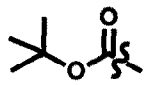
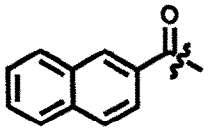
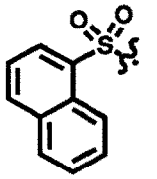
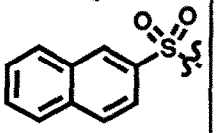
		CCK_B/Gastrin receptor (Mouse Stomach)		CCK-A receptor (Guinea-Pig Gallbladder)
No.		**p[A50]**	**α**	
1	Boc-CCK8 (27-33)	8.7	100	**p[A50]** = 8.8 α = 100
2	Boc-[Leu^{31}]-CCK4 (30-33)	8.6	100	**pK_B** = 5.1
3a	Boc-CCK_2 (32-33)	5.7	100	inactive (at 10^{-4}M)

a. p[A_{50}] represents the midpoint location parameter. α is the maximum acid secretion obtained at 10^{-5}M, expressed as a percentage of the maximum response to pentagastrin. (n = 4/6)

The observation that this particular dipeptide fragment possessed structural features sufficient for both recognition *and* stimulation of the gastrin/CCK_B receptor prompted the synthesis of a number of derivatives. A similar selectivity profile was observed for other N-protected derivatives of the dipepetide. Thus the tert-butyloxycarbonyl or Boc group in **3a** could be replaced with 2-naphthalenecarbonyl **4a** or naphthalenesulphonyl **5a** and **6a**[18] without altering the gastrin/CCK_B-receptor activity, suggesting that this property derived from the Asp-Phe-NH_2 moiety and was independent of the nature of the N-protecting group. Furthermore, each of these compounds was also inactive as tested at CCK_A receptors (Table 2.). However, when these same structural changes were made in conjuction with removal of the C-terminal amide, the compounds obtained were all inactive at gastrin/CCK_B receptors. On the other hand, investigation of the action of these same molecules on the guinea-pig gallbladder CCK_A-receptor assay established that the 2-naphthalene containing compounds **4b** and **6b** behaved as competitive antagonists at the CCK_A receptor with the sulphonamide **6b** being the more potent, while the Boc and 1-naphthalenesulphonyl analogues **3b** and **5b** respectively were inactive as tested. Thus, this series of compounds displayed a reversal of selectivity to those compounds with a C-terminal amide, in that they were now inactive at gastrin/CCK_B receptors and exhibited a range of activity at CCK_A receptors that is dependent on the type of N-protecting group.

Table 2. Agonist Concentration-Effect Curve Parameters for CCK_2 (32-33) Derivatives[19].



R	No.	X	CCK_B/Gastrin receptor[a] p[A50]	CCK_B/Gastrin receptor[a] α	CCK_A receptor[a] pK_B (± s.e)
	3a	$CONH_2$	5.7	100	i.a.
	3b	H	i.a.		i.a.
	4a	$CONH_2$	5.0	100	i.a.
	4b	H	i.a.		5.6±0.2
	5a	$CONH_2$	4.6	30	i.a.
	5b	H	i.a.		i.a.
	6a	$CONH_2$	5.6	96	i.a.
	6b	H	i.a.		6.5±0.1

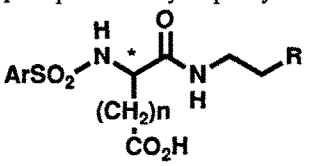
a. i.a. indicates inactive at the limits of solubility, usually 10^{-4}M. α is the maximum acid secretion obtained at 10^{-5}M, expressed as a percentage of the maximum response to pentagastrin. (n = 4/6)

Structure-activity studies based around the 2-naphthalenesulphonamide derivative **6b** established some of the requirements for CCK_A-receptor antagonism in this class of compounds (Table 3.). This property was insensitive to substitution on the naphthyl ring as shown by the sterically-demanding trisubstituted replacement **9**. However, the importance of the location of the aromatic system in **6b** was evident since insertion of an additional methylene **10** resulted in a loss in activity. Certain mononuclear aromatic sulphonamides retained activity, including 3,4-dichlorophenyl **14**. Of the phenylalkyl sulphonamides **15** to **17**, the phenethyl

derivative **16** alone retained significant activity, indicating that only in this example is the increased entropy, due to removal of an aromatic ring, offset by sufficiently good positioning of the remaining ring. This hypothesis was supported with the preparation of the styrene sulphonamide **18** which was as potent as the parent compound **6b**.

Affinity for the CCK_A receptor was found to be sensitive to both the stereochemistry at the amino acid α–carbon, and length of the carboxylic acid side chain. Changing from L-aspartic acid **6b** to the D-enantiomer **19**, or extending the side chain in the case of the L-glutamic acid derivative **20** resulted in a loss in affinity. Furthermore the D-glutamic acid derivative **21** was inactive as tested, in contrast to the trend observed for CR1505 and related compounds where a clear preference for derivatives of D-glutamic acid exists[4].

Table 3. Affinity Parameters at CCK_A receptors for Arylsulphonyl acidic amino acid aromatic amides[19].



No.	Ar	*	n	R	pK_B[a] (± s.e)
7	6-MeO-2-naphthyl-	S	1	phenyl-	6.6±0.2
8	5-Cl-6-MeO-2-naphthyl-	S	1	phenyl-	6.5±0.4
9	5-Cl-6,7-MeO_2-2-naphthyl-	S	1	phenyl-	6.2±0.3
10	2-naphthylmethyl-	S	1	phenyl-	5.4±0.2
11	phenyl-	S	1	phenyl-	i.a.
12	4-Me-phenyl-	S	1	phenyl-	i.a.
13	4-MeO-phenyl-	S	1	phenyl-	i.a.
14	3,4-Cl_2-phenyl-	S	1	phenyl-	5.8±0.3
15	benzyl-	S	1	phenyl-	i.a.
16	phenylethyl-	S	1	phenyl-	5.5±0.2
17	phenylpropyl-	S	1	phenyl-	i.a.
18	β-styryl-	S	1	phenyl-	6.3±0.2
19	2-naphthyl-	R	1	phenyl-	5.9±0.3
20	2-naphthyl-	S	2	phenyl-	6.1±0.2
21	2-naphthyl-	R	2	phenyl-	i.a.
22	2-naphthyl-	S	1	4-F-phenyl-	6.6±0.2
23	2-naphthyl-	S	1	4-MeO-phenyl-	6.0±0.3
24	2-naphthyl-	S	1	4-H_2NSO_2-phenyl-	5.0±0.3
25	2-naphthyl-	S	1	benzyl-	7.0±0.2

a. i.a. indicates inactive at the limits of solubility, usually 10^{-4}M. (n = 4/6)

A limited investigation with the introduction of substituents in the 4-position of the phenethyl aromatic ring showed that only in the case of the sulphonamide **24** was there a significant loss in activity. Although the preferred steric and electronic demands of this ring remain unclear, nonetheless, introduction of an additional methylene to give the phenylpropyl derivative **25** showed a substantial increase in affinity with respect to **6b**. This would presumably result in increased conformational space available to the phenyl aromatic ring, allowing a better interaction with the receptor, and contrasts with the apparent preference for conformational constraint around the naphthalene ring.

None of the examples listed in Table 3 showed activity in gastrin/CCK_B-receptor assays at the limits of their solubility emphasising the high CCK_A-receptor selectivity of these compounds.

The receptor specificity of the sodium salt of **6b**, 2-NAP, and its *in-vitro* characterisation as a competitive CCK_A-receptor antagonist has been established on bioassay in functional and radioligand binding assays and is reported elsewhere[20]. Moreover, the results obtained from the radioligand binding assays (CCK_A (guinea-pig pancreatic cells) $pK_i = 6.45 \pm 0.07$ (n=5), CCK_B (mouse cerebral cortex) $pK_i = 4.16 \pm 0.11$ (n=5)) are consistent with the affinity estimates derived from the functional studies. The relatively low Log P of this ligand with respect to other reported CCK_A-receptor antagonists identifies 2-NAP as a useful tool with which to investigate peripheral CCK_A receptors (Table 4). The efficacy of 2-NAP in man is currently being evaluated.

Table 4. Log P measurements for CCK_A-receptor antagonists[21].

Log P (chloroform/buffer (pH 7.4))

	254nm	280nm
2-NAP	-0.91	-0.91
CR1505	+0.47	+0.48
MK-329	+4.16	+4.10

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19. The compounds were synthesised by standard peptide coupling procedures using known protection-deprotection methods and will be reported in detail elsewhere. All new compounds gave satisfactory spectral and analytical data.

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21. 5mg of compound was shaken with ethanol-free chloroform (2mL) and Krebs-Henseleit buffer (pH 7.4) (2mL) and the mixture stirred for 30 min. The mixture was allowed to stand at room temperature for a further 30 min., the layers separated and filtered through a Millex-HV_{13} filter unit. Analysis was determined by HPLC using a Waters 710 WISP automatic injection system (C8 column 75%:25% acetonitrile/water with 0.1% acetic acid).