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SYNTHESIS AND SAR STUDY OF NOVEL CCK-B ANTAGONISTS

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Abstract: The structure activity relationship and an efficient synthesis of N,N-disubstituted amides of α-methyltryptophan via stable oxazolone derivatives **12a** and **12b** are described.

The neuropeptide cholecystokinin (CCK), a 33-amino acid polypeptide, was isolated from porcine intestine[1] and has been found in numerous molecular forms at various sites throughout the peripheral and central nervous systems in a number of mammalian species.[2,3] Various biological functions are attributed to this peptide including gall bladder contraction, pancreatic exocrine secretion, and neurotransmission and neuromodulation in the central nervous system.[4,5] The receptors for CCK have been classified into two subtypes according to their affinity for CCK fragments and their analogs.[6-9] CCK-A receptors are found predominantly in peripheral tissues such as pancreas and gall bladder. They have high affinity for the sulfated octapeptide (CCK-8S) and lower affinity for the corresponding desulfated fragment CCK-8d, CCK-4 and gastrin. Conversely, CCK-B receptors are widely distributed throughout the brain and exhibit high affinity for CCK-8s, CCK-4 and gastrin.

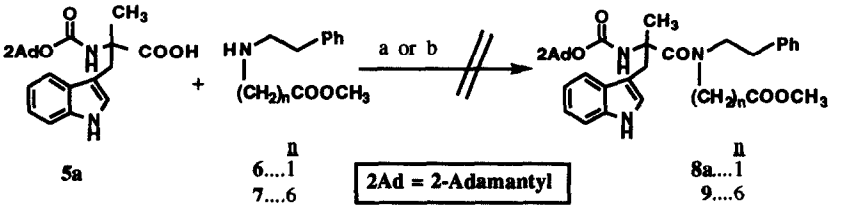
Recently, much attention has been focused on the development of potent and selective antagonists of the CCK-B receptor because of its putative role in nociception and anxiety. We have previously published rationally designed, potent and selective CCK-B receptor antagonists (**1a, 1b**, **2a**, and **2b**).[10,11] We have observed that the C-terminus of the 'dipeptoid' can tolerate considerable manipulation without significant loss of binding activity.[12] Furthermore, we have also shown that the carboxylic acid functionality on the C-terminus enhances both affinity and selectivity.[13] The carboxylic acid group is proposed to mimic the sidechain of Asp 32 in the C-terminal octapeptide of cholecystokinin, CCK-26-33 (sulfated).[14] This carboxylic acid moiety is free to explore a large volume of space, and it may serve as an accessory binding group to the receptor. Our earlier results, having a mobile sidechain with a terminal carboxylic moiety appended on either the α-carbon (**1a** and **1b**) or β-carbon (**2a** and **2b**) of the phenethylamide, have supported our strategy of designing a 'super ligand'.[10,11]

On the basis of these observations and in an attempt to eliminate the chiral center at the C-terminus as well as to stabilize the backbone amide to both base and enzymatic degradation[15], we decided to investigate a series of N,N-disubstituted amides with a carboxylic acid or its ester derivative at the terminus of the sidechain.

The required 2-adoc-α-methyltryptophans **5a** and **5b** were prepared as described previously.[11] The secondary amines **6**, **7**, **16** and **21** were prepared in variable yields by alkylation of the arylethylamines with the appropriate halo esters. Alkylation of phenethylamine with ethyl 4-bromobutyrate gave 1-(2-phenethyl)-2-pyrrolidinone. Therefore, the corresponding t-butyl ester was used to prepare the secondary amine **13**.

Initial approaches to prepare the targeted compounds **8a** and **9** by coupling of the acid **5a** with the secondary amines **6** and **7** using DCC/HOBT or CDI were unsuccessful even after

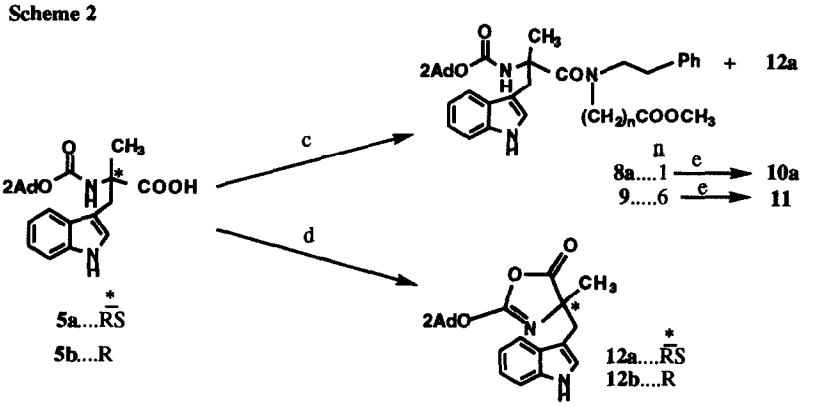
Scheme 1



(a) DCC, HOBT; n=1 (b) CDI; n=6

prolonged heating (Scheme 1). In both the cases, the secondary amines did not react with the activated ester. When BOP-Cl was used as a coupling agent[16], the products **8a** and **9** were obtained in low yields (35% and 25% respectively), and the major product isolated was the oxazolone **12a**. The oxazolones **12a** and **12b** were also prepared independently in quantitative yields by the reaction of BOP-Cl with the corresponding acids **5a** and **5b** in the presence of triethylamine at room temperature (Scheme 2). The mechanism presumably involves acylation

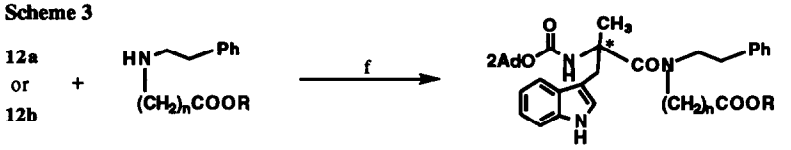
Scheme 2



(c) $PhCH_2CH_2NH(CH_2)_nCOOCH_3$, BOP-Cl, TEA, 0°C→ rt. (d) BOP-Cl, TEA, rt. (e) LiOH/ aq. Dioxane

at the oxygen of the urethane.[17] Subsequent reaction of **12a** and **12b** with the relatively unreactive secondary amines **6** and **13** provided the targeted amides **8a**, **8b** and **14** in 85%, 71% and 76% isolated yields. The methyl ester derivatives **8a** and **8b** were then saponified by LiOH

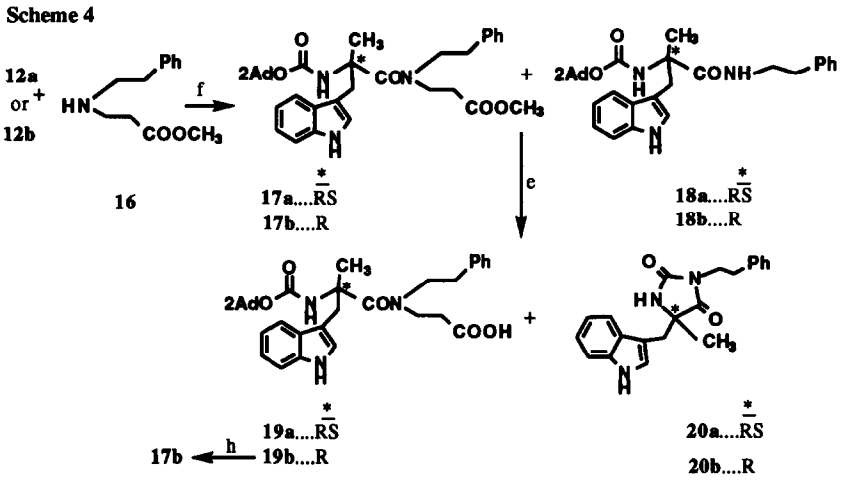
Scheme 3



(e) LiOH/aq. Dioxane (f) Toluene, reflux (g) HCOOH

in aqueous dioxane to yield the corresponding acids **10a** and **10b**. The t-butyl ester **14** was hydrolyzed by treatment with formic acid at room temperature to obtain the acid **15** (Scheme 3). In the case of the secondary amine **16**, the coupling reaction with the oxazolone **12a** gave an inseparable mixture of the desired product **17a** and by-product **18a** which upon hydrolysis afforded the acid **19a** and the hydantoin derivative **20a**. The presence of **18a** was confirmed by spectral analysis and by coinjecting a standard sample of **18a** with the mixture on HPLC. Additionally, the rapid cyclization of **18a** to the hydantoin **20a** under basic conditions has been published.[18] Similar results were observed in the coupling reaction of the same secondary amine

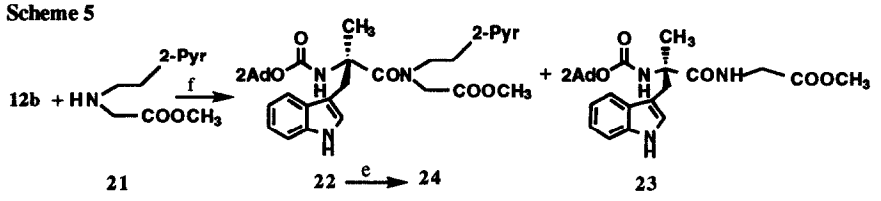
Scheme 4



(e) LiOH/aq. Dioxane (f) Toluene, reflux (h) HCl/Methanol

16 with the chiral oxazolone **12b** (Scheme 4). In the case of the secondary amine **21**, the coupling reaction gave a low yield of the desired product **22** along with **23**, apparently via a β-elimination under the reaction conditions (Scheme 5).

Scheme 5



(e) LiOH/aq. Dioxane (f) Toluene, reflux

The CCK receptor binding affinities of the compounds prepared[18] for this study are given in Table 2. The results show that compounds with a shorter sidechain terminating in a carboxylic acid functionality have better binding affinity and greater selectivity for the CCK-B receptor compared to the corresponding ester derivatives (**10a** vs. **8**, **19b** vs. **17b**, **24** vs. **22**). The binding affinity and selectivity for the CCK-B receptor also decrease with increasing length of the acid sidechain (**10b** vs **19b** vs **15**). These results suggest the importance of the optimal through-bond distance of the carboxylic acid group from the phenethyl backbone. This conclusion supports our previous finding regarding the SAR of compounds with the acid sidechain at the α- and β-positions of the phenethylamide.[13] Previously, the introduction of an acetic acid moiety onto the α-carbon of the phenethylamide was shown to cause a marked increase in the binding affinity (**1a** and **1b** vs. **3**) and selectivity (**1a** vs. **3**) for the CCK-B receptor. However, placement of an acetic acid moiety onto the amide nitrogen leads to only a two-fold improvement in the binding affinity (**10b** vs. **3**). Interestingly, in the case of the pyridylethyl analogs, the introduction of an acetic acid moiety significantly improves the binding affinity (**24** vs. **4**). One possible explanation for this improvement is that a hydrogen bond between the pyridyl nitrogen and the acid functionality may contribute to a favorable and stable configuration for interaction at the receptor. In general, we have observed no improvement in the binding affinities by appending a mobile sidechain with a terminal carboxylic acid moiety on to the amide nitrogen, contrary to the regioisomeric series (**1a** and **1b**, **2a** and **2b**). One of the reasons for the lack of improvement may be the existence of higher proportions of undesired rotomers due to restricted rotation of the amide bond for the disubstituted amide series.[20]

The compound **10b** was tested in the CCK-B receptor-rich neurons of the rat ventromedial hypothalamic nucleus (VMN)[12], and was found it to be an antagonist at the CCK-B receptor.

In conclusion, we have developed an efficient method to synthesize disubstituted amide derivatives of α-methyltryptophan via a stable oxazolone derivative. We have shown that these derivatives with terminal carboxylic acid functionality maintain potent binding affinities for the CCK-B receptor. We have also shown that proper placement of an acetic acid moiety at the C-terminus is critical for maintaining good binding affinity and selectivity for the CCK-B receptor.

Finally, combining the N-acetic acid with a C-terminal 2-(2-pyridylethyl) moiety afforded compound **24** which has excellent binding affinity and selectivity for the CCK-B receptor.

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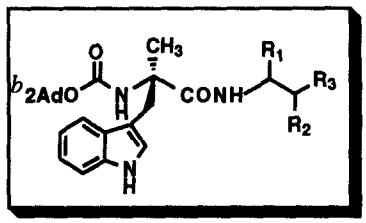
19. All new compounds gave satifactory spectral data and elemental analyses. ^{1}H NMR at room temperature of all the targeted compounds showed existence of rotomers.
20. We thank one of the reviewers for this suggestion.

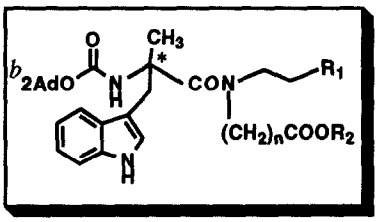
Table 1. CCK Receptor Binding Affinities of Reference Compounds[a]



COMPOUND	R_1	R_2	R_3	IC_{50} (nM) CCK-A	IC_{50} (nM) CCK-B	A/B
1a	(S) CH_2COOH	H	Ph	25.5	0.15	170
1b	(R)CH_2COOH	H	Ph	186	9.3	20
2a	H	(R)$NHCO(CH_2)_2COOH$	Ph	4300	1.7	2500
2b	H	(S)$NHCO(CH_2)_2COOH$	Ph	3100	34	72
3	H	H	Ph	377	48	7.9
4	H	H	2-Pyr	1846	15.5	119

[a] IC_{50} represents the concentration (nM) producing half-maximal inhibition of specific binding of [^{125}I] Bolton Hunter CCK-8 to CCK receptors in the mouse cerebral cortex (CCK-B) or the rat pancreas (CCK-A). The values given are the geometrical mean of at least three separate experiments. [b]2Ad refers to 2-adamantyl.

Table 2. CCK Receptor Binding Affinities of Targeted Compounds[a]



COMPOUND	*	n	R_1	R_2	IC_{50} (nM) CCK-A	IC_{50} (nM) CCK-B	A/B
10a	RS	1	Ph	H	>1000[c]	40.9[d]	24.4
10b	R	1	Ph	H	1079	25.7	41.9
8	RS	1	Ph	Me	2330	532	4.4
19a	RS	2	Ph	H	672	64.2	10.5
19b	R	2	Ph	H	527	36.8	14.3
17b	R	2	Ph	Me	1980	113	17.5
15	R	3	Ph	H	564	80.2	7.0
11	RS	6	Ph	H	>1000	348	2.9
9	RS	6	Ph	Me	6320	177	35.7
24	R	1	2-Pyr	H	337	2.31	145.9
22	R	1	2-Pyr	Me	2520	90.9	27.7

[a] Binding affinities and [b]2Ad as defined in footnote Table 1. [c] represents the value of a single experiment. [d] represents the geometrical mean of two separate experiments.