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L-TRYPTOPHAN UREA AMIDES AS NK1/ NK2 DUAL ANTAGONISTS

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Abstract: We report that a systematic modification of an NK_1 receptor selective antagonist resulted in the identification of novel compounds, 4c and 4d, with high affinity for both NK_1 and NK_2 receptors. © 1998 Elsevier Science Ltd. All rights reserved.

The neuropeptides, Substance P (SP) and Neurokinin A (NKA), function as neurotransmitters in the peripheral and central nervous system, where they predominantly interact with the Neurokinin 1 (NK₁) and Neurokinin 2 (NK₂) receptors, respectively.¹ The release of SP and NKA stored in afferent nerves in the airways by various stimuli has been shown to cause excessive mucus secretion, airway constriction, and plasma extravasation. Since these effects are very similar to the typical clinical symptoms of asthma, it has been suggested that both SP and NKA might be involved in the pathology of asthma.² Thus, a dual NK₁/NK₂ antagonist might offer a novel method for the treatment of asthma. When this work was initiated, a macrocyclic peptide FK-224, a dual antagonist of the NK₁ and NK₂ receptors (NK₁ IC₅₀ = 37 nM, NK₂ IC₅₀ = 72 nM), was reported to provide some protection against bradykinin induced bronchoconstriction in asthmatic patients.³ Recently, dual NK₁ and NK₂ antagonists were obtained by modification of the NK₂ selective compound SR48968.⁴ In this paper, we report on the design and synthesis of L-tryptophan based NK₁/NK₂ dual antagonists derived from the NK₁ selective L-tryptophan benzyl ester 1 reported previously from these laboratories.⁵



L-Tryptophan benzyl esters have been reported to be selective NK₁ antagonists. 3,5-Disubstitutions on the benzyl group, especially by CF₃ (**1**, NK₁ IC₅₀ = 1.6 nM, NK₂ IC₅₀>5 μ M), can markedly enhance the activity for NK₁ receptors. It was also found that a variety of substituents on the amino acid nitrogen were tolerated for high NK₁ receptor affinity, for example, the acetamide, methyl carbamate and *N*-methyl urea (**2**, NK₁ IC₅₀ = 103 nM, NK₂ IC₅₀ > 5 μ M) were all NK₁ antagonists.⁵ However, on the C-terminus of the molecule, replacement of the benzyl ester with the tertiary benzyl amide (**3**, NK₁ IC₅₀ > 1.1 μ M, NK₂ IC₅₀ > 5 μ M) resulted in compounds with poor affinity for NK₁ and NK₂ receptors. In comparing 1 with the NK₂ selective antagonist SR48968,⁶ it occurred to us that the 3,4-dichloro phenyl and the indolymethyl moieties might interact with the receptors in a similar fashion. Also, the phenyl group of the amide in compound 2 and the phenyl group of benzamide in SR48968 are connected by four single bonds to the indolylmethyl or 3,4-dichloro phenyl. Because of the flexibility of the substituents on the nitrogen for NK₁ activity in the tryptophan ester series, we rationalized that by incorporating the piperidine type functionality of SR48968 into the tryptophan ester or amide system to form an urea might result in compounds possessing both NK₁ and NK₂ receptors affinity .

The synthesis of the L-tryptophan urea amides was straightforward and is shown in Scheme I. For easy modification of the N-terminal urea, Method A was used. N-Boc-L-Trp was coupled with the appropriate amine under the standard coupling conditions (EDC/HOBT) and the Boc was removed by treatment with trifluoroacetic acid. The resulting amine was sequentially reacted with 1,1'-carbonyldiimidazole (CDI) and then selected substituted piperidines to afford the urea analogs. Alternatively, the process was reversed for more convenient C- terminal modification (Method B). Thus, urea formation between L-Trp-OBn and the piperidines, followed by deprotection of the benzyl ester via catalytic hydrogenation afforded the common carboxylic acid intermediate, which was converted to the desired products by coupling with various amines under standard conditions.



Reagents: (a) EDC, HOBt, NMM, R₁R₂NH, CH₂Cl₂; (b) TFA, Anisole; (c) CDI, Azacycles, THF; (d) H₂, Pd/C, EtOH

Binding affinities for these compounds on NK₁ were determined using ¹²⁵I-Tyr⁸-SP at a concentration equivalent to its K_d (0.1 nM) on the human NK₁ receptor, stably expressed in CHO cell as previously described.⁷ A similar protocol was used for NK₂ activity where cloned human NK₂ receptors expressed in CHO cells and ¹²⁵I -NKA as ligand were employed. A series of compounds with various substituted piperidines on the urea portion was prepared while the *N*-methyl benzyl amide moiety was retained at the C-terminus of the molecules (**Table I**). Incorporation of the piperidine moiety from SR48968 into the L-Trp urea provided a compound (4a) with moderate affinity for NK₁ and NK₂ receptors (IC₅₀ NK₁/NK₂ = 77/928 nM respectively). Further modification of the piperidine with a 4- spiroindeno or 4-spiroindano piperidine resulted in 4b (68/18 nM) and 4c (56/27 nM) with improved NK₂ binding while still retaining NK₁ activity of 4a. Compound 4d, incorporating a 4-spiroindolinosulfonamide piperidine in the urea portion, was the most potent

and balanced antagonist in this series with an IC_{50} of 14 nM on NK₁ and 24 nM on NK₂. The R-enantiomer of 4d, (4d'), (164/577 nM) was prepared from D-Trp and it was found to have substantially less affinity for both NK₁ and NK₂ receptors. Thus, the S-stereochemistry was preferred.

Table I Affinity of L-Trp Ureas 4 for the Cloned Human NK₁ and NK₂ Receptors.⁸

		N Ph Me NH	ACNH		N N N	MsN	>
4		Ra	Rb	Rc	Rd		
IC ₅₀ (nM)						IC ₅₀ (nM)	
Compound	R	hNK1	hNK ₂	Compounds	R	hNK ₁	hNK ₂
4a	Ra	77	928	4d	Rd	14	24
4b	Rb	68	18	4d' (<i>R</i>)	Rd	164	577
4c	Rc	56	27				

After modification of the urea portion, the SAR on the amide portion was studied (**Table II**). To test whether an amide was necessary for dual activities, two esters, **5a** and **5b**, were prepared. With no substitutions on the benzyl group, **5a** (23/4500 nM) maintained affinity on the NK₁ receptor but lost NK₂ activity compared to the amide **4d** (14/24 nM). As expected, inclusion of the 3,5-di-CF₃ substituents as in **5b** (0.58/>1000 nM) resulted in a much higher affinity at NK₁ receptor but poorer affinity at NK₂ receptor. In the amide series, **5c** (10/1400 nM) with 3,5-diMe substituents and **5d** (1.0/4200 nM) with 3,5-diCF₃ substituents also showed enhanced NK₁ receptor affinity and diminished activity at NK₂ receptor compared with **4d**.

Table II Affinity of L-Trp Ureas 5 and 6 for the Cloned Human NK1 and NK2 Receptors



					IC ₅₀ (nM)		
Compound	Х	R_1	R ₂	n	hNK1	hNK ₂	
5a	0		Н	1	23	4500	
5b	0		CF ₃	1	0.58	>1000	
4d	Ν	Me	Н	1	14	24	
5e	Ν	Me	Me	1	10	1400	
5d	Ν	Me	CF ₃	1	1.0	4200	
6a	Ν	Н	Н	0	39%@3000	2100	
6b	Ν	Н	Н	1	227	14	
6c	Ν	Н	Н	2	332	211	
4c	Ν	Me	Н	1	56	27	
6d	Ν	Et	Н	1	242	139	

Since an unsubstituted benzyl amide was required for dual affinity, we then investigated the distance between the phenyl and the amide nitrogen as well as the substitutions on the nitrogen in the spiroindane series (6). Among the N-H derivatives, a single methylene was found to be optimal (compare 6b with 6a and 6c, Table II). The preferred nitrogen substitution was found to be $R_1 = Me$ (compare 4c with 6b and 6d, Table II).

We also studied the replacement of the indolylmethyl group with a phenethyl or benzyloxy methyl by starting with L-homophenylalanine or O-benzyl-L-serine, respectively, using a similar synthetic route. These replacements resulted in the loss of affinity at both receptors.

Table III Affinity of Ureas 7 for the Cloned Human NK₁ and NK₂ Receptors.

0			IC ₅₀ (nM)		
MSN N. H. L.	Compounds	R	hNK1	hNK ₂	
O R Me	7a	PhCH ₂ CH ₂ -	1100	166	
<u>`_</u> / 7	7b	PhCH ₂ OCH ₂ -	480	258	

In conclusion, systematic modification of an NK_1 receptor selective antagonist resulted in the identification of novel compounds, **4c** and **4d**, with high affinity for both NK_1 and NK_2 receptors.

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References and Notes

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- 8. Each reported IC_{50} s is the average of three independent determinations.