N-Acyl-L-tryptophan Benzyl Esters: Potent Substance P Receptor Antagonists

Angus M. MacLeod,* Kevin J. Merchant, Margaret A. Cascieri, Sharon Sadowski, Elzbieta Ber, Christopher J. Swain, and Raymond Baker

Merck, Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, U.K., and Merck Research Laboratories, Rahway, New Jersey 07065

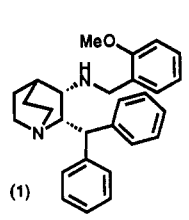
Received May 14, 1993

Substance P is an undecapeptide belonging to the tachykinin family of neurotransmitters which are related by a homologous C-terminal sequence:

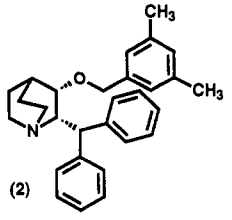
Phe-xxx-Gly-Leu-Met-NH_2

The mammalian tachykinins (substance P and the decapeptides neurokinin A and neurokinin B) bind to a class of neurokinin (NK) receptors NK_1, NK_2, and NK_3 to which substance P binds with a selectivity in the order $NK_1 > NK_2 > NK_3$. Since being first described by von Euler and Gaddum in 1931,[1] substance P has been the subject of considerable investigation and has been implicated in the transmission of pain signals and the initiation of inflammatory responses. Because of these effects, substance P antagonists may have potential use in analgesia[2] and as novel anti-inflammatory agents for use in the treatment of migraine[3] and rheumatoid arthritis.[4] Until recently, however, it has not been possible to fully evaluate this potential because of the absence of suitable pharmacological tools.

Since the disclosure[5] in 1991 of CP 96,345 (1), the first non-peptide substance P antagonist, several reports of small molecule antagonists have appeared.[6] Recent studies in our laboratories[7] have demonstrated that high affinity for the NK_1 receptor is retained in analogues of CP 96,345 in which the benzylamine moiety is replaced by a benzyl ether, with optimal activity observed in 3,5-disubstituted derivatives 2. Upon screening the Merck sample collection to identify novel compounds in this area, we found that *N*-ethyl-L-tryptophan benzyl ester (3) is a weak inhibitor ($IC_{50} = 3.8\mu M$) of substance P binding to the human NK_1 receptor. In this paper we describe studies based on this lead compound resulting in the development of simple tryptophan derivatives[8] that are highly potent NK_1 antagonists (see Table I).



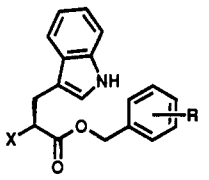
(1)



(2)

The compounds in this study were prepared by alkylation (Scheme I) of the cesium salt of *N*-Boc-L-tryptophan in DMF with various substituted benzyl halides to give esters 4. Removal of the Boc group from 4 with ethereal HCl gave primary amines, which were alkylated to the *N,N*-dimethylamines or *N,N,N*-trimethylammonium quaternary salts. *N*-Acetyl derivatives 7 were similarily prepared from *N*-acetyl-L-tryptophan.

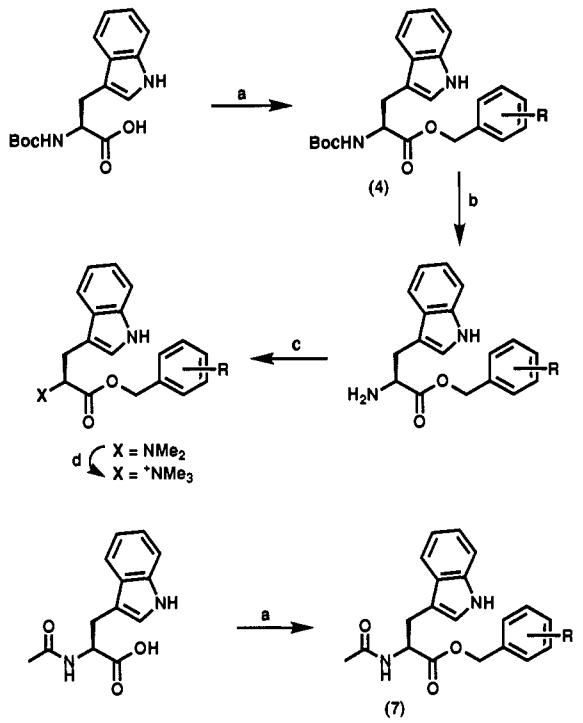
Table I. Human NK_1 Receptor Binding



compd	X	R	mp (°C)	IC_{50} (nM)[a]
CP 96,345				0.2
3	NHEt	H		3800 ± 235
5	NH_2	H		>10000
4a	NHBoc	H	132–133	413 ± 281
4b	NHBoc	2-OMe	132–133	280 ± 99
4c	NHBoc	3,5-$(CH_3)_2$	152–153	133 ± 33
6a	NH_2	3,5-$(CH_3)_2$	213–214	1533 ± 462
6b	NMe_2	3,5-$(CH_3)_2$	129–130	553 ± 41
6c	NMe_3	3,5-$(CH_3)_2$	164–165	125 ± 19
7a	NHAc	3,5-$(CH_3)_2$	145–146	67 ± 10
7b	NHAc	3,5-$(CF_3)_2$	147–148	1.6 ± 0.7

[a] Displacement of ^{125}I-labeled substance P from the cloned receptor expressed in CHO cells. Data are reported as the mean ± SD for $n = 3$ determinations.

Scheme I[a]



[a] Reagents: (a) cesium carbonate, DMF, and then substituted benzyl bromide; (b) HCl, methanol; (c) CH_2O, MeOH, $NaCNBH_4$, CH_3CO_2H; (d) CH_3I, acetone.

Binding affinities for these compounds were measured in radioligand binding experiments determined by the displacement of ^{125}I-labeled substance P from the cloned human NK_1 receptor, stably expressed in CHO cells.[9] In the synthesis of the unsubstituted benzyl ester 5, it was found that the *tert*-butyl carbamate intermediate 4a had a greater than 9-fold higher affinity for the receptor than the screening lead (3) or the primary amine 5. Substitution on the benzyl ester with an *o*-methoxy group (4b), as found in CP-96,345, resulted in a small increase in binding affinity while 3,5-disubstitution with methyl (4c) (as found in the benzyl ether analogs of CP-96,345) gave a further enhancement in potency to 133 nM. Removing the *tert*-butyl carbamate from 4c led to a 12-fold loss in affinity

0022-2623/93/1836-2044$04.00/0

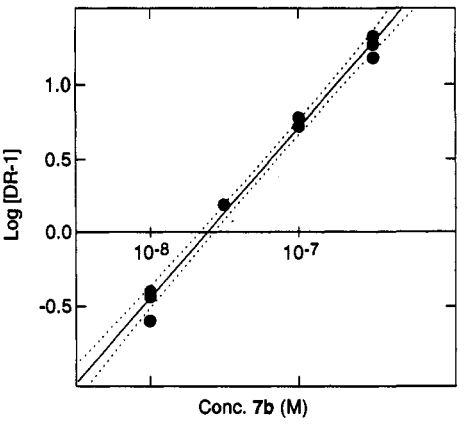


Figure 1. Schild analysis of compound **7b**.

(**6a**), some of which was recovered by progressive methylation of the primary amine to the dimethylamine **6b** and quaternary ammonium salt **6c**. Altering the carbamate group to an *N*-acetyl side chain improved binding 2-fold (**7a**), but the most dramatic effect on affinity (**7b**) came from introducing electron withdrawing character into the benzyl ester substituents, while retaining lipophilicity at the *meta* positions.

Using the 3,5-bis(trifluoromethyl)benzyl ester **7b**,[10] inhibition of substance P induced inositol phosphate accumulation in CHO cells expressing the human NK_1 receptor was assayed as previously described.[9] Increasing concentrations of **7b** progressively increased the apparent EC_{50} for substance P without altering the maximal response to the agonist. Schild analysis of these data was linear with a slope of 1.15, indicating that the compound functions as a competitive antagonist of substance P activity (Figure 1). The K_b for **7b** in this system was 25 nM. At this stage, it is not known precisely how compounds of this class, or other non-peptide antagonists, relate to the endogenous peptide in its binding to the receptor. Finally, all of the compounds described above were highly selective for NK_1, with affinities weaker than 5 μM at the human NK_2 and NK_3 receptors. These compounds represent a novel structural class of substance P receptor antagonists and are highly potent leads for further optimisation.

Detailed structure–activity and functional studies with **7b** will be reported in subsequent papers.

Acknowledgment. We thank Fred Brookfield and Sarah Hardwicke for synthetic chemistry support.

References

(1) von Euler, U. S.; Gaddum, J. H. An unidentified Depressor Substance in Certain Tissue Extracts. *J. Physiol.* **1931**, *72*, 74–87.

(2) Otsuko, M.; Yanagisawa, M. Effect of a Tachykinin Antagonist on a Nociceptive Reflex in the Isolated Spinal Cord Tail Preparation of the New-born Rat. *J. Physiol. (London)* **1988**, *395*, 255–270.

(3) Moskowitz, M. A. Neurogenic Versus Vascular Mechanisms of Sumatriptan and Ergot Alkaloids in Migraine. *Trends Pharmacol. Sci.* **1992**, *13*, 307–311.

(4) Lotz, M.; Carson, D. A.; Vaughan, J. H. Substance P Activation of Rheumatoid Synoviocytes: Neural Pathway in Pathogenesis of Arthritis. *Science* **1987**, *235*, 893–895.

(5) (a) Snider, R. M.; Constantine, J. W.; Lowe, J. A., III; Longo, K. P.; Lebel, W. S.; Woody, H. A.; Drozda, S. E.; Desai, M. C.; Vinick, F. J.; Spencer, R. W.; Hess, H. J. A Potent Non-peptide Antagonist of the Substance P (NK_1) Receptor. *Science* **1991**, *251*, 435–437. (b) Lowe, J. A., III; Drozda, S. E.; Snider, R. M.; Longo, K. P.; Zorn, S. H.; Morrone, J.; Jackson, E. R.; McLean, S.; Bryce, D. K.; Bordner, J.; Nagahisa, A.; Kanai, Y.; Suga, O.; Tsuchiya, M. The discovery of (2S,3S)-*cis*-2-(Diphenylmethyl)-N-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine as a Novel, Nonpeptide Substance P Antagonist. *J. Med. Chem.* **1992**, *35*, 2591–2600.

(6) (a) Desai, M. C.; Lefkowitz, S. L.; Thadeio, P. F.; Longo, K. P.; Snider, R. M. Discovery of a Potent Substance P Antagonist: Recognition of the Key Molecular Determinant. *J. Med. Chem.* **1992**, *35*, 4911–4913. (b) Lawrence, K. B.; Venepalli, B. R.; Appell, K. C.; Goswami, R.; Logan, M. E.; Tomczuk, B. E.; Yanni, J. M. Synthesis and Substance P Antagonist Activity of Naphthimidazolium Derivatives. *J. Med. Chem.* **1992**, *35*, 1273–1279. (c) Garret, C. G.; Carruette, A.; Fardin, V.; Moussaoui, S.; Peyronel, J-P.; Blanchard, J-C.; Laduron, P. M. Pharmacological Properties of a Potent and Selective Nonpeptide Substance P Antagonist. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10208–10212. (d) Schilling, W.; Bittiger, H.; Brugger, F.; Criscione, L.; Hauser, K.; Ofner, S.; Olpe, H. R.; Vassout, A.; Veenstra, S. Approaches Towards the Design and Synthesis of Nonpeptidic Substance-P Antagonists. Xth International Symposium on Medicinal Chemistry, Basel, September 1992, Abstract ML-11.3. (e) Oury-Donat, F.; Lefevre, I. A.; Gauthier, T.; Emonds-Alt, X.; Le Fur, G.; Soubrie, Ph. SR 140333, A Novel and Potent Non-peptide Antagonist of the NK_1 Receptor. *Neuropeptides* **1993**, *24*, 233.

(7) Seward, E.; Swain, C. J.; Merchant, K. J.; Owen, S. N.; Sabin, V.; Cascieri, M. A.; Sadowski, S.; Strader, C.; Baker, R. Quinuclidine-Based NK-1 Antagonists I: 3-Benzyloxy-1-azabicyclo[2.2.2]octanes. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1361–1366.

(8) Di- and tripeptide NK_1 antagonists that contain a D-tryptophan residue have been developed from (D-Pro^4,D-$Trp^{7,9,10}$,Phe^{11})SP_{4-11}: Morimoto, M.; Murai, M.; Maeda, Y.; Hagiwara, D.; Miyake, H.; Matsuo, M.; Fujii, T. FR 113680: a Novel Tripeptide Substance P Antagonist with NK_1 Receptor Selectivity. *Br. J. Pharmacol.* **1992**, *106*, 123–126; Fujii, T.; Murai, M.; Morimoto, H.; Maeda, Y.; Yamaoka, M.; Hagiwara, D.; Miyake, H.; Ikari, N.; Matsuo, M. Pharmacological Profile of a High Affinity Dipeptide NK_1 Receptor Antagonist, FK888. *Br. J. Pharmacol.* **1992**, *107*, 785–789.

(9) Cascieri, M. A.; Ber, E.; Fong, T. M.; Sadowski, S.; Bansal, A.; Swain, S.; Seward, E.; Frances, B.; Burns, D.; Strader, C. D. Characterisation of the Binding of a Potent, Selective Radioiodinated Antagonist to the Human Neurokinin-1 Receptor. *Mol. Pharmacol.* **1992**, *42*, 458–465.

(10) **Synthesis of *N*-Acetyl-L-tryptophan 3,5-Bis(trifluoromethyl)-benzyl Ester (7b).** *N*-Acetyl-L-tryptophan (4.9 g) was dissolved in methanol (100 mL) and water (10 mL). Cesium carbonate (2.6 g) in water (50 mL) was added, the solvent was removed *in vacuo*, and the residue was azeotroped with anhydrous toluene (2 × 100 mL). 3,5-Bis(trifluoromethyl)benzyl bromide (6.16 g) in dimethylformamide (10 mL) was added to a solution of the cesium salt in dimethylformamide (100 mL) and the reaction was stirred for 16 h. The solvent was removed *in vacuo* and the residue partitioned between ethyl acetate and water. The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo* to give a solid which was recrystallized from ethyl acetate/petroleum ether to yield **7b** (3.7g): mp 147–148 °C; $[\alpha]^{25}_D$ −4.2°; ^1H NMR (360 MHz, $CDCl_3$) δ 8.01 (1H, s), 7.83 (1H, s), 7.61 (1H, s), 7.51 (1H, d, J = 8 Hz), 7.32 (1H, d, J = 8 Hz), 7.17 (1H, t, J = 7 Hz), 7.09 (1H, t, J = 7 Hz), 6.91 (1H, d, J = 2 Hz), 5.98 (1H, s), 5.13 (1H, d, J = 13 Hz), 5.06 (1H, t, J = 13 Hz), 4.96 (1H, t, J = 6 Hz), 3.31 (2H, m), 1.98 (1H, s).