Identification of L-Tryptophan Derivatives with Potent and Selective Antagonist Activity at the NK_1 Receptor

Angus M. MacLeod,* Kevin J. Merchant, Frederick Brookfield, Fintan Kelleher, Graeme Stevenson, Andrew P. Owens, Christopher J. Swain, Margaret A. Cascieri, Sharon Sadowski, Elzbieta Ber, Catherine D. Strader, D. Euan MacIntyre, Joseph M. Metzger, Richard G. Ball, 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

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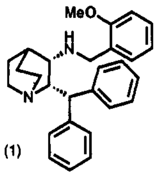
As part of a program of screening the Merck sample collection, *N*-ethyl-L-tryptophan benzyl ester was identified as a weak antagonist at the substance P (NK_1) receptor. Structure–activity studies showed that the indole ring system could be replaced by 3,4-dichlorophenyl, α- or β-naphthyl, or benzthiophene with retention or only small loss of affinity. It was found that acylation of the tryptophan nitrogen gave compounds with higher affinity than *N*-ethyl or other basic amines. Optimization of substitution on the benzyl ester led to the identification of the 3,5-bis(trifluoromethyl)benzyl ester of *N*-acetyl-L-tryptophan **26** as a potent and selective substance P receptor antagonist. Compound **26** blocked substance P induced dermal extravasation *in vivo* and was the most potent compound from this structurally novel class of antagonists which further adds to the diversity of small molecules that bind to the (NK_1) receptor.

Introduction

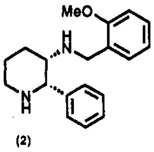
Since the discovery[1] of substance P (SP) more than 60 years ago, the pharmacology of this neurotransmitter has been studied in great detail. It has been established[2] that SP is involved in the transmission of pain signals and that SP antagonists can block the nociceptive effect induced by capsaicin. SP is involved in inflammatory processes and has been implicated[3] in the pathogenesis of rheumatoid arthritis. There is also evidence[4] that inflammation of the dura caused by neurogenic SP release may be the source of migraine headaches. Consequently, there is considerable interest in this neurotransmitter system as a point of pharmacological intervention in the therapy of common clinical conditions. SP was characterized[5] at the molecular level in 1970 and shown to be an undecapeptide with sequence Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2. It belongs to the tachykinin family of neuropeptides which includes neurokinins A and B, related by the common C-terminal sequence Phe-xxx-Gly-Leu-Met-NH_2. These peptides bind to a series of G-protein coupled neurokinin receptors, NK_1, NK_2, and NK_3, which have selectivity for SP, NKA, and NKB, respectively.

Until recently, only peptide agonists and antagonists at the NK_1 receptor were available with limited opportunity to evaluate their clinical potential because of low oral bioavailability. The importance of the disclosure[6] of CP-96,345 (1), the first non-peptide SP antagonist, is reflected in the large number of studies that have been carried out subsequently using this compound in models of pain and inflammation. Some antinociceptive activities[7] of CP-96,345 have been attributed to action at the calcium channel[8] and also are seen with its receptor-inactive enantiomer. However, 1, but not its inactive enantiomer, inhibits plasma extravasation[9] in the guinea pig induced by either exogenous substance P or capsaicin and exhibits analgesic activity[10] in acetic acid induced abdominal stretching in mice.

It has now been shown[11] that the structure of CP-96,345 can be simplified, with full retention of activity, by removing an ethylene bridge from the quinuclidine and modification of the benzhydryl substituent to a phenyl ring (CP-99,994, **2**). Recent SAR studies on CP-96,345 in our laboratories[12] have demonstrated that high affinity for the human NK_1 receptor is retained in analogues of 1 in which the benzylamine moiety is replaced by a benzyl ether, with optimal activity observed in 3,5-disubstituted derivatives (**3**).



(1)



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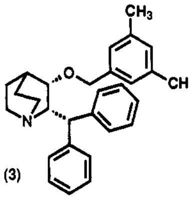

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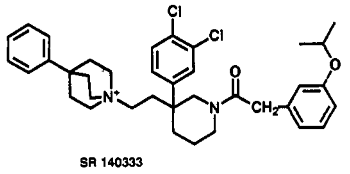
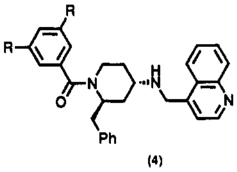


SR 140333



(4)

Several reports of other small molecule antagonists with high affinity for the human NK_1 receptor have now appeared including the quaternary ammonium quinuclidine derivative SR 140333[13] and a series[14] of substituted benzoyl piperidines (**4**). Naphthimidazolium derivatives (**5**),[15] which are structurally quite diverse from CP-96,345, have moderate affinity at the rat NK_1 receptor while RP-67,580,[16] which shares the diphenylmethyl and *o*-methoxyphenyl moieties of CP-96,345, is a potent rat NK_1 antagonist. Some progress has also been made in the development of non-peptide antagonists starting from the endogenous neurotransmitter.[17] The tripeptide FR113680 was designed[18] from (D-Pro^4,D-$Trp^{7,9,10}$,Phe^{11})SP_{4-11} and refined[19] to a dipeptide FK888 with high affinity for NK_1 and selectivity with respect to NK_2 and NK_3.

As part of a screening effort to identify novel compounds in this area we found that *N*-ethyl-L-tryptophan benzyl ester (**6**) is a weak inhibitor (IC_{50} 3.8 μM) of substance P

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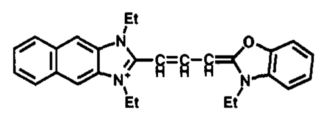

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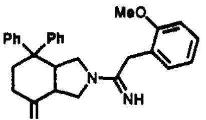
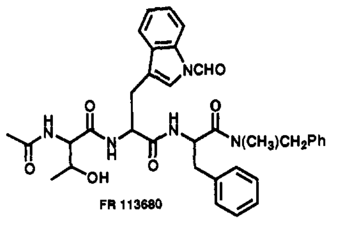
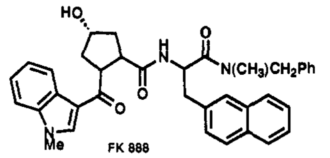


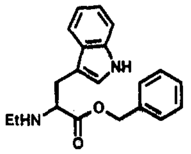
RP-67,580



FR 113680



FK 888



(6)

binding to the human NK$_1$ receptor. Although the ester group of this compound is an obvious liability in terms of *in vivo* activity, particularly after oral dosing, this compound was perceived as a useful starting point for a novel chemical series of substance P antagonists. Our first objective in this area was to determine whether a substantial improvement in *in vitro* binding affinity could be achieved by structural modification of **6**, before addressing the issue of ester stability. In this paper we describe studies based on this lead compound resulting in the development of tryptophan derivatives that are highly potent NK$_1$ antagonists.[20]

Chemistry

The compounds of this study were prepared by alkylation (Scheme 1) of the cesium salt of *N*-Boc-L-tryptophan in DMF with various substituted benzyl halides to give esters (**7**). Removal of the Boc group from **7** with methanolic HCl gave primary amines (**8**) which were either reductively alkylated or converted to nonbasic compounds by reaction with an acid chloride, isocyanate, or chloroformate. Related compounds were made in the same way from other amino acids.

Biology

A stable CHO cell line expressing the human NK$_1$ receptor was used[21] to determine binding affinity of compounds prepared in this series with [^{125}I]Tyr^8-substance P as radioligand. Inhibition of substance P induced inositol phosphate accumulation in CHO cells expressing the human NK$_1$ receptor was assayed as previously described.[21] Substance P induced plasma extravasation assays were performed in guinea pigs injected with substance P in the dorsal skin. Inhibition of extravasation was measured by leakage of Evans Blue dye after administration of the test compound.

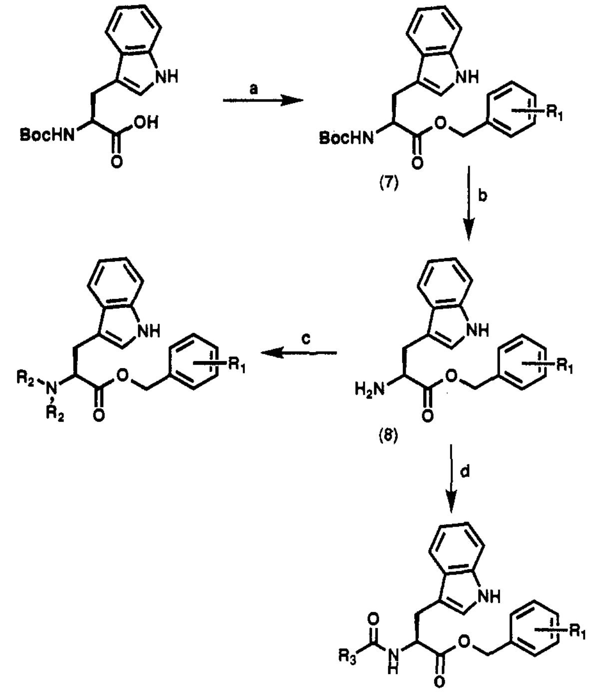
Results and Discussion

It emerged at an early stage that basicity in the α-amino ester was disadvantageous since the *tert*-butyl carbamate synthetic intermediate **7a** was more potent (Table 1) than the amines **6** and **8a**. Analogues of **7a** incorporating substituents in the aryl ring chosen by analogy with CP-96,345 (2-OMe) or the related series of quinuclidine ethers 3[12] (3,5-dimethyl) produced a 2–4-fold increase in affinity (**7b,c**). Structure–activity around substitution on the tryptophan nitrogen was developed with the 3,5-dimethylbenzyl ester (Table 2). N,N-Dimethylation (**10**) produced a modest increase in affinity while quaternization (**11**) improved potency 12-fold over the primary amine **8b**. A greater enhancement in activity was found by acylation to the acetamide **12** and comparable compounds were found in the methyl carbamate **13** and *N*-methylurea **14**. Introduction of aromatic character with the benzamide **15a** further improved affinity and preparation of the D-enantiomer (**15b**) of this compound established a 250-fold enantiomeric specificity in the ligand-receptor interaction. This observation is in contrast to the tripeptide NK$_1$ antagonist[18] FR 113680 in which the tryptophan residue has the D-configuration.

Substitution on the benzyl ester aryl ring was further exemplified by monosubstitution around the ring with chloro, methoxy, and trifluoromethyl groups (Table 3). Despite the improved activity with an *o*-methoxy substituent (**7b**), no relationship was found to the SAR of the benzylamine moiety[9a] of CP-96,345. With the *m*-trifluoromethyl variation (**23**) highlighted as the best of this group, the 3,5-bis(trifluoromethyl)benzyl ester **25** was synthesized and displayed greatly enhanced potency compared to the dimethyl analogue (**15a**). This substantial increase in affinity was even greater when the same modification was applied to the acetamide derivative **12** (Table 2) to give **26** (Table 4) with IC$_{50}$ 1.6 nM.

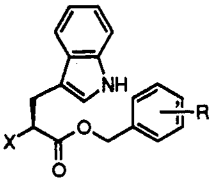
A survey (Table 4) of possible replacements for indole in compounds of this type showed that this ring system is not as critical as the 3,5-bis(trifluoromethyl)phenyl ring in achieving good NK$_1$ binding affinity. While indazole **28** was a poor mimic of indole, the racemic benzo[*b*]thiophene **27** was only 5-fold lower in affinity than **26**. Both α- and β-naphthyl compounds **29** and **30** retained good affinity compared to **12** with the latter marginally more active. Employing the same substitution pattern,

Scheme 1[a]



[a] Reagents: (a) cesium carbonate, DMF then substituted benzyl bromide; (b) HCl, methanol; (c) CH_2CO, MeOH, $NaCNBH_4$, CH_3CO_2H for **10**, followed by CH_3I, acetone for **11**; (d) RCOCl, RNCO or RO_2CCl, pyridine.

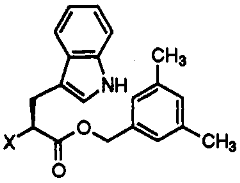
Table 1. Human NK$_1$ Receptor Binding



compound	X	R	analysis	mp (°C)	IC$_{50}$ (nM)^a
CP-96,345					0.4 ± 0.1
6	NHEt	H			3800 ± 235
8a	NH$_2$	H			>10000
7a	NHBoc	H	$C_{23}H_{26}N_2O_4$	132–133	413 ± 281
7b	NHBoc	2-OMe	$C_{24}H_{28}N_2O_5$	132–133	280 ± 99
7c	NHBoc	3,5-(Me)$_2$	$C_{25}H_{30}N_2O_4 \cdot 0.25H_2O$	152–153	133 ± 33

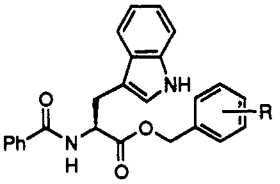
^{a} In all tables IC$_{50}$ refers to displacement of ^{125}I-labeled substance P from the cloned NK$_1$ receptor expressed in CHO cells. Data are reported as the mean ± SD for $n = 3$ determinations.

Table 2. Variation of Nitrogen Substituent



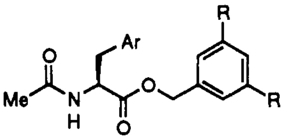
compound	X	analysis	mp (°C)	IC$_{50}$ (nM)
7c	NHBoc	$C_{25}H_{30}N_2O_4 \cdot 0.25H_2O$	152–153	133 ± 33
8b	NH$_2$	$C_{20}H_{22}N_2O_2 \cdot HCl \cdot 0.25H_2O$	213–214	1533 ± 462
10	NMe$_2$	$C_{22}H_{26}N_2O_2 \cdot HCl \cdot 0.67H_2O$	129–130	553 ± 41
11	NMe$_3$	$C_{23}H_{26}N_2O_2I$	164–165	125 ± 19
12	NHCOCH$_3$	$C_{22}H_{24}N_2O_3 \cdot 0.25H_2O$	145–146	67 ± 10
13	NHCO$_2$Me	$C_{22}H_{24}N_2O_4$	128–129	87 ± 12
14	NHCONHMe	$C_{22}H_{26}N_3O_3 \cdot 0.33H_2O$	66–68	103 ± 26
15a (L)	NHCOPh	$C_{27}H_{26}N_2O_3 \cdot 0.25H_2O$	133–134	22 ± 3
15b (D)	NHCOPh	$C_{27}H_{26}N_2O_3$	131–132	5500 ± 1500

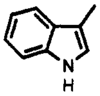
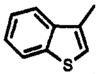
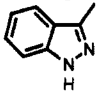
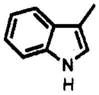
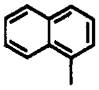
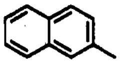
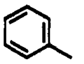
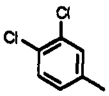
Table 3. Variation of Aromatic Substituent



compound	R	analysis	mp (°C)	IC$_{50}$ (nM)
15a	3,5-(Me)$_2$	$C_{27}H_{26}N_2O_3$	133–134	22 ± 3
16	2-Cl	$C_{25}H_{21}ClN_2O_3 \cdot 0.67H_2O$	151–152	600 ± 100
17	3-Cl	$C_{25}H_{21}ClN_2O_3$	146–147	1050 ± 50
18	4-Cl	$C_{25}H_{21}ClN_2O_3$	119	243 ± 104
19	2-OMe	$C_{26}H_{24}N_2O_4 \cdot 0.5H_2O$	65–66	>100
20	3-OMe	$C_{26}H_{24}N_2O_4$	96–97	760 ± 140
21	4-OMe	$C_{26}H_{24}N_2O_4$	142–143	5500 ± 500
22	2-CF$_3$	$C_{26}H_{21}F_3N_2O_3$	117	183 ± 12
23	3-CF$_3$	$C_{26}H_{21}F_3N_2O_3 \cdot 0.25H_2O$	118	62 ± 6
24	4-CF$_3$	$C_{26}H_{21}F_3N_2O_3$	136–137	2150 ± 650
25	3,5-(CF$_3$)$_2$	$C_{27}H_{20}F_6N_2O_3$	182–184	2.7 ± 0.2

Table 4. Indole Replacements



compound	Ar	R	analysis	mp (°C)	IC$_{50}$ (nM)
26 (L)		CF$_3$	$C_{22}H_{18}F_6N_2O_3$	147–148	1.6 ± 0.7
27 (±)		CF$_3$	$C_{22}H_{17}F_6NO_3S$	129–130	8 ± 2
28 (±)		CF$_3$	$C_{21}H_{17}F_6N_3O_3$	120	197 ± 5
12 (L)		CH$_3$	$C_{22}H_{24}N_2O_3 \cdot 0.25H_2O$	145–146	67 ± 10
29 (L)		CH$_3$	$C_{24}H_{26}NO_3$	136–140	153 ± 20
30 (L)		CH$_3$	$C_{24}H_{25}NO_3$	96–97	138 ± 82
31 (L)		CH$_3$	$C_{20}H_{23}NO_3$	97–100	433 ± 85
32 (±)		CH$_3$	$C_{20}H_{21}Cl_2NO_3$	118–119	30 ± 8

the simple 3,4-dichlorophenyl derivative **32** was 2-fold more potent than **12** with the chlorine substituents contributing a 10-fold increase to the affinity of the parent phenylalanine **31**.

Conformational analysis of either **12** or **26** by random generation of conformers followed by energy minimization within SYBYL indicated a preference for structures in which a π–π interaction[22] can be achieved between the indole and 3,5-disubstituted aryl ring. The global minimum energy conformation found for **26** had these two rings disposed in an offset face-to-face configuration, similar to that found for the aryl rings in CP-96,345 and CP-99,994. In the crystal structure of **12** (Figure 1) the indole ring is orthogonal to the aryl ring but at a distance (8.2 Å between the centroids of the two phenyl rings) where no interaction takes place. In the unit cell, however, two close energetically favorable *inter*molecular edge-to-face aromatic associations are present which would be expected to influence the conformation seen in the solid phase. Because of disorder in the trifluoromethyl groups, it was not possible to obtain a well refined crystal structure of compound **26**. Comparison of energy-minimized conformations for ester **26** and the quinuclidine-based benzyl ether **3** shows (Figure 2) that an overlay of the common disubstituted phenyl rings can be achieved simultaneously with superimposition of the indole ring and the benzhydryl phenyl ring of **3** that is conserved in CP-99,994. Both **26** and **3** have several rotatable bonds, and there are other, higher energy, conformers where the overlay of these rings can be achieved. As with all studies of flexible molecules, the preferred structures in the solid or gas phase may be

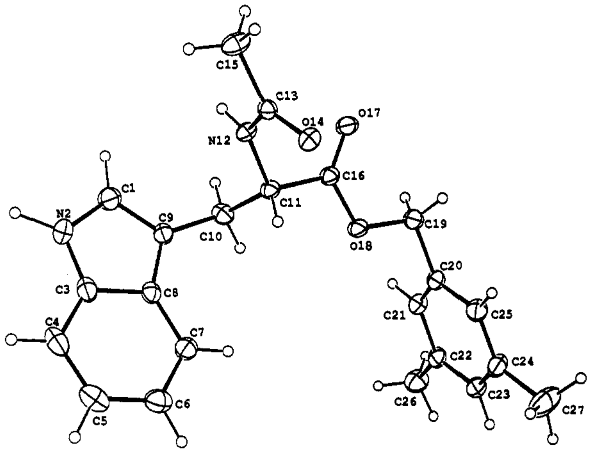


Figure 1. X-ray crystal structure of 12 with 20% probability elipsoids. Hydrogen atoms have been drawn at an arbitrary size and the numbering is as used in the determination.

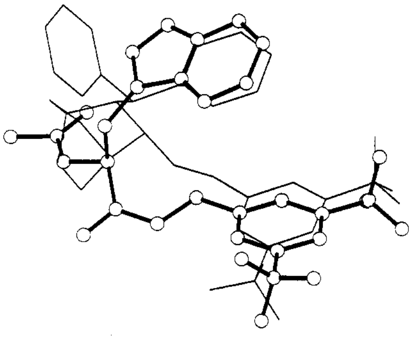


Figure 2. Superposition of low energy conformations of tryptophan ester 26 (ball and stick representation) with quinuclidine ether 3. Hydrogen atoms are omitted for clarity.

quite different than those induced by ligand–receptor binding energies at the active site of the receptor; high-affinity NK_1 ligands with fewer degrees of rotational freedom are required to better define the biorelevant conformation of these compounds. Confirmation that these structural classes share common binding sites has come from mutagenesis studies[23] which show that the 3,5-disubstituted phenyl rings in both series interact with His265 in the 6th putative transmembrane spanning region of the receptor while the indole of 26 and one of the benzhydryl phenyl rings of 3 bind to His197 in the 5th helical domain. It is notable that, while both classes of compounds behave as competitive antagonists, the mutation of either of these two histidine residues does not affect binding of substance P itself, indicating that the non-peptide antagonists occupy a volume of space in the receptor which is either partly or fully filled when the agonist binds, but using interactions which are not required by the agonist.

Selectivity for the NK_1 receptor was assessed by screening compounds against cloned human NK_2 and NK_3 receptors stably expressed in CHO cells using [^{125}I]-neurokinin A and [^{125}I]Bolton–Hunter labeled eledoisin as radioligands, respectively. Affinity for the other neurokinin receptors was greater than 5 μM (IC_{50}) for all of the compounds described in this study. The quinuclidine antagonists (e.g., CP-96,345) are selective for the human NK_1 receptor with substantially lower affinity[24] for the rat homologue. The same was found to be true for this new series of antagonists with 26 giving an IC_{50} of 192 nM at the rat receptor. As previously reported,[20] compound 26 increased the apparent EC_{50} for substance P induced inositol phosphate synthesis in CHO cells expressing the human NK_1 receptor without altering the maximal response to substance P. Schild analysis of the data indicated that the compound functions as a competitive antagonist of substance P activity.

The same compound was tested *in vivo* for its ability to inhibit substance P induced plasma extravasation in the guinea pig. Test compounds were administered either by oral or intraperitoneal dosing 1 h before a challenge with substance P injected into the skin of the animals. Leakage of plasma into the skin surrounding the sites of injection was determined by measuring levels of Evans Blue dye which had been introduced intravenously prior to the agonist challenge. In this model **26** inhibited extravasation with ID_{50} 8 mg/kg after administration ip while activity was much reduced after oral dosing with 22% inhibition observed at 30 mg/kg.

Conclusion

The compounds described in this paper represent an interesting new class of substance P antagonists with high *in vitro* binding affinity. While *in vivo* activity was demonstrated for compound **26** after systemic dosing, the substantially lower potency after oral administration is not unexpected with compounds which contain a potentially biologically labile ester group. Despite this, these compounds constitute an attractive lead series for further study, and approaches toward metabolically more stable analogues will be the subject of future reports.

Experimental Section

Melting points were determined with a Büchi capillary melting point apparatus and are uncorrected. NMR spectra were recorded at 360 MHz on a Bruker AM360 instrument. The term "dried" refers to drying of an organic phase over anhydrous sodium sulfate and then filtering, and organic solvents were evaporated on a Büchi rotary evaporator at reduced pressure. Optical rotations were measured at the sodium D line (589 nM) using a Perkin-Elmer 241 polarimeter. Column chromatography was carried out on silica gel (Merck Art 7734). Petroleum ether refers to petroleum ether with bp 60–80 °C. Elemental analyses were determined by Butterworth Laboratories Ltd., Teddington, England.

The following preparations serve to exemplify the methods used to synthesize compounds discussed in the text above. Compounds not specifically detailed may be prepared by analogy with these methods.

***N*-α-Boc-L-tryptophan 3,5-Dimethylbenzyl Ester (7c).** *N*-α-Boc-L-tryptophan (7.6 g, 25mmol) was dissolved in MeOH (100 mL) and water (10 mL). Cesium carbonate (4.05 g, 12.4 mmol) in water (50 mL) was added, the solvent was removed *in vacuo*, and the residue was azeotroped with anhydrous DMF (2 × 100 mL). 3,5-Dimethylbenzyl bromide (5.0 g, 25.3 mmol) in DMF (10 mL) was added to a solution of the cesium salt in DMF (100 mL), and the reaction was stirred for 16 h. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc and water. The organic phase was dried and evaporated to give a solid which was recrystallized from EtOAc/petroleum ether to yield the title compound as a white solid (6.8 g, 65%): mp 152–153 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (1H, s), 7.54 (1H, d, J = 8 Hz), 7.32 (1H, d, J = 8 Hz), 7.16 (1H, t, J = 7 Hz), 7.09 (1H, t, J = 7 Hz), 6.95 (1H, s), 6.64 (3H, s), 5.09–5.07 (1H, m), 5.00 (2H, m), 4.70–4.67 (1H, m), 3.29–3.28 (1H, m), 2.29 (6H, s), 1.42 (9H, s). Anal. ($C_{25}H_{30}N_2O_4\cdot0.25H_2O$) C, H, N.

L-Tryptophan 3,5-Dimethylbenzyl Ester (8b). Compound 7c (1.0 g, 2.4 mmol) was dissolved in dry THF (20 mL) to which was added saturated methanolic HCl (10 mL), and the solution was left to stand for 16 h. The solvent was removed *in vacuo* and the residue recrystallized from EtOH/Et_2O to give 0.71 g (82.5%) of **8b**: mp 213–214 °C; ^{1}H NMR (d_6-DMSO) δ 11.09 (1H, s), 8.64 (1H, s), 7.51 (1H, d, J = 7 Hz), 7.38 (1H, d, J = 7 Hz), 7.20 (1H, d, J = 2 Hz), 7.10 (1H, t, J = 7 Hz), 6.98 (1H, t, J = 7 Hz), 6.94 (1H, s), 6.76 (2H, s). Anal. ($C_{20}H_{22}N_2O_2\cdot HCl\cdot0.25H_2O$) C, H, N.

N-Acetyl-L-tryptophan 3,5-Dimethylbenzyl Ester (12). Compound **8b** (0.5 g, 1.4 mmol) in dry pyridine (0.5 mL) was treated with acetic anhydride (0.5 mL) for 16 h. EtOAc was added and the solution washed with 5 N HCl, brine, and water. The organic phase was dried and the solvent removed *in vacuo*. Chromatography on silica gel using EtOAc/petroleum ether (3:2) gave **12** as a white solid (0.17 g, 33%): mp 145–146 °C; ^1H NMR ($CDCl_3$) δ 8.19 (1H, s), 7.51 (1H, d, J = 7 Hz), 7.33 (1H, d, J = 7 Hz), 7.18 (1H, t, J = 7 Hz), 7.09 (1H, t, J = 7 Hz), 6.97 (1H, s) 6.87 (2H, s), 6.77 (1H, d, J = 2 Hz), 6.03 (1H, d, J = 8 Hz), 5.06–4.97 (3H, m), 3.37–3.26 (2H, m), 2.30 (6H, s), 1.94 (3H, s). Anal. ($C_{22}H_{24}N_2O_3 \cdot 0.25H_2O$) C, H, N.

N-Acetyl-L-tryptophan 3,5-Bis(trifluoromethyl)benzyl Ester (26). This was prepared from the cesium salt of N-acetyltryptophan and 3,5-bis(trifluoromethyl)benzyl bromide by a method analogous to that described for **7c**, **8b**, and **12** and crystallized from EtOAc/petroleum ether: mp 147–148 °C; ^1H NMR ($CDCl_3$) 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 (3H, s). Anal. ($C_{22}H_{18}F_6N_2O_3$) C, H, N.

N-Benzoyl-L-tryptophan 3,5-Dimethylbenzyl Ester (15a). Via the method used to prepare **12**, benzoyl chloride (500 mg, 3.6 mmol) and amino ester **8b** (500 mg, 1.4 mmol) gave **15a** (0.21 g, 35%): mp 133–134 °C; $[\alpha]^{21}_D$ −24.0° (c = 1, MeOH); ^1H NMR ($CDCl_3$) δ 8.10 (1H, s), 7.67 (1H, d, J = 7 Hz), 7.53 (1H, d, J = 7 Hz), 7.49–7.25 (4H, m), 7.17 (1H, t, J = 7 Hz), 7.05 (1H, t, J = 7 Hz), 6.97 (1H, s), 6.89 (2H, s), 6.82 (1H, d, J = 2 Hz), 6.68 (1H, d, J = 8 Hz), 5.18 (1H, m), 5.06 (2H, s), 3.45 (2H, m), 2.3 (6H, s). Anal. ($C_{27}H_{26}N_2O_3 \cdot 0.25H_2O$) C, H, N.

N-Benzoyl-D-tryptophan 3,5-dimethylbenzyl ester (15b) was prepared in the same way: mp 131–132 °C; $[\alpha]^{21}_D$ +23.3° (c = 2, MeOH); NMR identical to the L-enantiomer. Anal. ($C_{27}H_{26}N_2O_3$) C, H, N.

3,5-Dimethylbenzyl 2-(N,N-Dimethylamino)-3-(3-indolyl)propionate Hydrochloride (10). To a solution of compound **8b** (500 mg, 1.4 mmol) in MeOH (30 mL) was added sodium cyanoborohydride (220 mg, 3.5 mmol) and acetic acid (1 mL). The reaction was cooled to 0 °C and formaldehyde solution (38% w/v, 300 mg) in MeOH (20 mL) was added over 0.25 h. The reaction was stirred for 2 h, then the solvents were removed *in vacuo*, and the residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution. The organic extract was dried and evaporated to yield an oil which was purified by column chromatography on silica using EtOAc/petroleum ether (4:1). The oil thus obtained was treated with methanolic HCl and the solvent removed to yield **10** as a white solid (95 mg, 17%): mp 129–130 °C; ^1H NMR (d_6-DMSO) δ 11.11 (1H, s), 7.64 (1H, d, J = 7 Hz), 7.39 (1H, d, J = 7 Hz), 7.16 (1H, d, J = 2 Hz), 7.11 (1H, t, J = 7 Hz), 7.01 (1H, t, J = 7 Hz), 6.88 (1H, s), 6.55 (2H, s), 4.95 (1H, d, J = 12 Hz), 4.81 (1H, d, J = 12 Hz), 4.38–3.28 (2H, m), 2.91 (6H, m), 2.17 (6H, s). Anal. ($C_{22}H_{26}N_2O_2 \cdot HCl \cdot 0.6H_2O$) C, H, N.

3,5-Dimethylbenzyl 3-(3-Indolyl)-2-(N,N,N-trimethylammonio)propionate Iodide (11). A solution of compound **10** (500 mg, 1.4 mmol) in acetone (1 mL) and Et_2O (2.0 mL) was treated with MeI (1.14g, 8 mmol) for 16 h. The resulting precipitate was filtered and dried to yield **11** (350 mg, 51%): mp 164–165 °C; ^1H NMR (d_6-DMSO) δ 11.07 (1H, s), 7.56 (1H, d, J = 7 Hz), 7.41 (1H, d, J = 7 Hz), 7.18–7.03 (3H, m), 6.67 (1H, s), 6.42 (2H, s), 4.90 (1H, d, J = 12 Hz), 4.75 (1H, d, J = 12 Hz), 4.62–4.58 (1H, m), 3.66–3.61 (1H, m), 3.31 (1H, s), 3.38–3.29 (1H, m), 2.14 (6H, s). Anal. ($C_{23}H_{30}N_2O_2I$) C, H, N.

3,5-Dimethylbenzyl 2-(3-Methylureido)-3-(3-indolyl)propionate (14). Compound **8b** (1.0 g, 2.8 mmol) suspended in THF (10 mL) was treated with Et_3N (0.38 mL, 2.8 mmol) and CH_3NCO (0.19 mL, 3.3 mmol) for 1 h. The solvent was removed *in vacuo*, and the residue in EtOAc was washed with dilute HCl, water, and $NaHCO_3$ solution, dried, and concentrated. The residual solid was recrystallized from EtOAc/petroleum ether to yield **14** (0.82 g, 77%): mp 66–68 °C; ^1H NMR ($CDCl_3$) δ 7.99 (1H, s), 7.52 (1H, d, J = 8 Hz), 7.30 (1H, d, J = 8 Hz), 7.16 (1H, t, J = 8 Hz), 7.08 (1H, t, J = 8 Hz), 6.96 (1H, s), 6.88 (2H, s), 6.76 (1H, s), 5.01 (2H, s), 4.83 (1H, m), 3.26 (2H, d, J = 5 Hz), 2.65 (3H, s), 2.30 (6H, s). Anal. ($C_{22}H_{25}O_3N_3 \cdot 0.3H_2O$) C, H, N.

3,5-Dimethylbenzyl 2-[(Methoxycarbonyl)amino]-3-(3-indolyl)propionate (13). By a similar procedure to the previous example compound **8b** and CH_3OCOCl gave **13** which was crystallized from EtOAc/petroleum ether: mp 128–129 °C; ^1H NMR ($CDCl_3$) δ 8.04 (1H, s), 7.52 (1H, d, J = 8.0 Hz), 7.32 (1H, d, J = 8 Hz), 7.18 (1H, t, J = 8 Hz), 7.09 (1H, t, J = 8 Hz), 6.95 (1H, s), 6.83 (2H, s), 5.25 (1H, d, J = 7.5 Hz), 5.00 (2H, dd, J = 12 Hz), 4.73 (1H, m), 3.65 (3H, s), 3.29 (2H, d, J = 5 Hz), 2.29 (6H, s). Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

The remaining compounds were prepared from the appropriate amino acids by the methods described for compounds **12** and **26**.

(±)-3,5-Bis(trifluoromethyl)benzyl 2-acetamido-3-(3-indazolyl)propionate (28): mp 120 °C dec; ^1H NMR (d_6-DMSO) δ 8.75 (1H, bs), 7.67 (1H, s,), 7.57 (2H, s), 7.40 (1H, m), 7.25 (3H, m), 7.14 (1H, m), 5.11 (3H, m), 3.66 (1H, m), 3.45 (1H, m), 1.99 (3H, s). Anal. ($C_{21}H_{17}F_6N_3O_3$) C, H, N.

(±)-3,5-Bis(trifluoromethyl)benzyl 2-acetamido-3-(3-benzo[*b*]thienyl)propionate (27): mp 129–130 °C; ^1H NMR ($CDCl_3$) δ 7.82 (1H, m), 7.75 (1H, m), 7.72 (1H, m), 7.60 (2H, s), 7.36 (2H, m), 7.25 (1H, s), 6.00 (1H, s), 5.12 (1H, d, J = 8.0 Hz), 5.06 (1H, d, J = 7.0 Hz), 5.04 (1H, d, J = 7.0 Hz), 5.02 (1H, d, J = 8.0 Hz), 3.44 (1H, t, J = 6.0 Hz), 1.97 (3H, s). Anal. ($C_{22}H_{17}F_6NO_3S$) C, H, N.

(±)-3,5-Dimethylbenzyl 2-acetamido-3-(3,4-dichlorophenyl)propionate (32): mp 118–119 °C; ^1H NMR ($CDCl_3$) δ 7.24 (1H, d, J = 8 Hz), 7.12 (1H, d, J = 2 Hz), 7.00 (1H, s), 6.91 (1H, s), 6.80 (1H, dd, J = 8, 2 Hz), 6.01 (1H, d, J = 7 Hz), 5.06 (2H, dd, J = 12, 12 Hz), 4.89 (1H, m), 3.07 (2H, m), 2.33 (6H, s), 2.00 (3H, s). Anal. ($C_{20}H_{21}Cl_2NO_3$) C, H, N.

N-Acetyl-L-phenylalanine 3,5-dimethylbenzyl ester (31): mp 97–100 °C; ^1H NMR ($CDCl_3$) δ 8.09 (1H, d, J = 8 Hz), 7.85 (1H, d, J = 7 Hz), 7.75 (1H, d, J = 8 Hz), 7.53–7.45 (2H, m), 7.34 (1H, t, J = 7 Hz), 7.25 (1H, t, J = 9 Hz), 6.93 (1H, s), 6.74 (2H, s), 5.07 (1H, bd, J = 7 Hz), 5.00 (1H, d, J = 12 Hz), 4.91 (1H, d, J = 12 Hz), 4.78-4.76 (1H, m), 3.72–3.47 (2H, m), 2.28 (6H, s), 1.40 (3H, s). Anal. ($C_{20}H_{23}NO_3$) C, H, N.

3,5-Dimethylbenzyl (2*S*)-2-acetamido-3-(1-naphthyl)propionate (29): mp 136–140 °C; ^1H NMR ($CDCl_3$) δ 8.10 (1H, d, J = 8.2 Hz), 7.85 (1H, d, J = 7.6 Hz), 7.75 (1H, d, J = 8.1 Hz), 7.53–7.45 (2H, m), 7.31 (1H, t, J = 7.1 Hz), 7.15 (1H, d, J = 6.5 Hz), 6.94 (1H, s), 6.73 (1H, s), 6.02 (1H, bd), 5.06 (1H, q, J = 6.35 Hz), 4.94 (2H, AB q, J = 12.0 Hz), 3.58 (2H, d, J = 6.2 Hz), 2.23 (6H, s), 1.92 (3H, s). Anal. ($C_{24}H_{25}NO_3$) C, H, N.

3,5-Dimethylbenzyl (2*S*)-2-acetamido-3-(2-naphthyl)propionate (30): mp 96–97 °C; ^1H NMR ($CDCl_3$) δ 7.80–7.78 (1H, m, Ar), 7.71–7.67 (2H, m, Ar), 7.47–7.42 (3H, m, Ar), 7.13 (1H, d, J = 10.0 Hz), 6.97 (1H, s, Ar), 6.88 (2H, s, Ar), 5.92 (1H, d, J = 7.5 Hz), 5.06 (2H, d, J = 3.1 Hz), 5.03–4.98 (1H, m), 3.29 (2H, d, J = 5.8 Hz), 2.28 (6H, s), 1.98 (3H, s). Anal. ($C_{24}H_{25}NO_3$) C, H, N.

Molecular Modeling. Conformational studies on compounds **12** and **26** (both as *S*-enantiomers) were carried out using the random search facility in SYBYL (version 5.5; Tripos Associates Inc.) with generation of 250 structures for each compound followed by full-energy minimization for each structure. All freely rotating bonds were searched with minimization by the Powell method in vacuo using the Tripos force field parameters, with Gasteiger–Hückel charges. The global minimum energy conformers found for both compounds had the indole and 3,5-disubstituted phenyl rings in an offset face-to-face configuration, and there were several other edge-to-face or face-to-face structures lower in energy than the lowest energy extended conformation. For **26**, the lowest energy conformer with these rings remote from each other was 5.7 kcal/mol higher in energy than the global energy minimum.

The SYBYL forcefield is not explicitly parameterized for $\pi-\pi$ interactions but these are accounted for in the van der Waals and electrostatic terms in the calculation. We have done studies with two benzene molecules minimized in an offset face-to-face orientation which in SYBYL are calculated to have energy 5 kcal/mol lower than the two molecules in isolation. This compares with 2.1 kcal/mol stabilization energy calculated for this system in the detailed studies of Jorgensen *et al.* (ref 22).

X-ray crystallography of 12: $C_{22}H_{24}N_2O_3$, M_r = 364.45, monoclinic, $P2_1$, a = 8.398(2), b = 8.1301(7), c = 14.968(1) Å, b = 99.64 (1)°, V = 1007.5 Å^3, Z = 2, D_x = 1.201 g cm^{-3}, monochromatized radiation λ(Cu Kα) = 1.541 84 Å, μ = 0.61 mm^{-1}, $F(000)$ = 388, T = 296 K. Data collected[25] on a Rigaku AFC5R diffractometer to a 2θ limit of 145° with 2062 observed,

$I > 3\sigma(I)$, reflections out of 2188 measured. Structure solved by direct methods using *SHELXS*-86[26] and refined using full-matrix least squares on F. Final agreement statistics are $R = 0.045$, $R_w = 0.056$, $S = 3.65$, $(\Delta/\sigma)_{max} = 0.3$. Weighting scheme is $1/\sigma^2(F)$. Maximum peak height in final difference Fourier map 0.21(6) e Å^{-3}. All calculations performed on a Sun Microsystems computer using *SDP-Plus*[27] software. Positional and thermal parameters as well as bond distances and angles are available as supplementary material.

In the crystal, two close edge-to-face associations, one aryl–aryl and one aryl–indole, are observed. One molecule has an ortho phenyl proton orthogonal to the plane of the indole ring of a second molecule, at a distance of 2.81 Å from the plane of the indole and pointing close to the center of the six-membered ring. The angle between the normals to the phenyl and indole rings is 81.98°. The other ortho proton of the same phenyl ring in this first molecule is 2.79 Å from the phenyl ring of a symmetry-related copy of itself with the normals to these two rings at an angle of 70.34°. A stereopair image of the unit cell showing these interactions is included in the supplementary material.

Substance P Induced Dermal Inflammation in the Guinea Pig. Male Dunkin Hartley guinea pigs were anaesthetised with Ketamine (25 mg/kg) and Acepromazine (2.5 mg/kg). The dorsal hair was shaved, and Evans Blue dye (0.5 mL; 2.5 g/100 mL in saline) was injected iv. After 10 min, substance P (0.5 pmol in 0.1% HSA saline) was injected intradermally, and exposure to the agonist continued for 1 h before sacrificing the animals by exposure to CO_2 gas. The injection sites on the dorsal surface were removed using 6-mm punch biopsies and the Evans Blue dye extracted by incubation overnight at 45 °C in formamide (0.5 mL). The extent of plasma extravasation was assessed by comparing the OD (at 650 nm) of the tissue extract to that of a known volume of plasma from the same animal. Test compounds were administered either ip or orally 1 h before substance P challenge.

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Supplementary Material Available: Details of the X-ray crystal structure determination for compound 12, including interatomic distances and angles and positional and thermal parameters, a stereopair drawing of the unit cell in the crystal structure of 12, together with perspective views of the two edge-to-face aromatic interactions (13 pages). Ordering information is given on any current masthead page.

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