Synthesis and Biological Evaluation of NK_1 Antagonists Derived from L-Tryptophan

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The 3,5-bis(trifluoromethyl)benzyl ester of *N*-acetyl-L-tryptophan (**3**), which was derived from the screening lead *N*-ethyl-L-tryptophan benzyl ester, has been used as a starting point to identify high-affinity substance P receptor antagonists with improved *in vivo* activity. Altering the ester moiety to an amide or ether led to a substantial loss in binding affinity, but conversion to a ketone provided compounds with affinity comparable to the equivalent esters. A homochiral synthesis of the key intermediate amino ketone **15** was developed which allows its preparation on a large scale. From this intermediate a range of amine-containing acylamino derivatives were prepared with affinity optimized in the morpholinylbutyramide **16l** which has an IC_{50} of 0.17 nM at the human NK_1 receptor. In addition to improving affinity, the amino group also provided aqueous solubility for a number of these derivatives. When tested *in vivo* the quinuclidine derivative L-737,488 (**16i**) was found to be an orally active (ID_{50} = 1.8 mg/kg) inhibitor of substance P-induced dermal extravasation in the guinea pig.

Introduction

Substance P is an 11-amino acid peptide,[1] with the sequence Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2, which has been implicated in a variety of disease states including rheumatoid arthritis,[2] migraine headaches,[3] and asthma[4] and is involved in the transmission of pain signals.[5] It belongs to a class of structurally related peptide neurotransmitters collectively known as the tachykinins that bind to the neurokinin receptors NK_1, NK_2, and NK_3. Substance P binds to all three of these receptors but with highest affinity toward the NK_1 subtype. The recent identification[6] of CP-96,345 (**1**), the first nonpeptide substance P antagonist, has made possible investigations into the pharmacology of the neurokinins that were not feasible with peptide antagonists and has confirmed the wide potential utility of this class of compounds. For example, it has been demonstrated[7] from studies performed in ferrets that some substance P antagonists are highly effective antiemetics and may be useful in alleviating Cisplatin- or radiation-induced emesis in the treatment of cancer, as well as sickness caused by other emetogens.

In addition to its NK_1 activity, CP-96,345 has been shown[8] to bind with high affinity to [^{3}H]diltiazem binding sites on the L-type calcium channel, and this may be responsible for some, but not all, of the functional effects seen with this compound. However, in an extension[9] of the medicinal chemistry of **1**, the piperidine-based compound CP-99,994 (**2**) has been developed as another high-affinity NK_1 ligand but with substantially reduced calcium channel binding. Several other structurally diverse series of nonpeptide substance P antagonists have now been reported including esters of *N*-acetyl-L-tryptophan,[10] with high affinity at the human NK_1 (hNK_1) receptor, which we developed from a lead identified through screening the Merck sample collection. The 3,5-bis(trifluoromethyl)benzyl ester **3** was shown to be a potent competitive substance P antagonist with an IC_{50} of 1.6 nM at the human NK_1 receptor. Compound **3** was active *in vivo*, after intraperitoneal dosing, in suppression of substance P-induced dermal extravasation in the guinea pig but was substantially less potent when dosed orally. The poor oral activity of these compounds was attributed to the likely rapid enzymatic hydrolysis of the biologically labile ester of **3**, and our first approach to improving oral activity was to replace the ester with a stable bioisostere. In this paper we describe the synthesis and structure−activity of amide, ether, and ketone analogues of these esters and demonstrate that the ketones have receptor binding affinities equivalent to the esters, with oral activity in animal models of inflammation. In addition, we have identified an auxiliary amine binding site which leads to enhanced affinity and allows for improved aqueous solubility in this class of compounds.

(1) CP-96,345 (2) CP-99,994

(3)

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Scheme 1

^{a} Reagents and conditions: (a) $LiAlH_4$, THF, then Boc_2O, DMAP, CH_3CN; (b) NaH, DMF, 3,5-dimethylbenzyl bromide; (c) HCl, MeOH; (d) Ac_2O, pyridine.

Chemistry

Ether derivatives of L-tryptophan were prepared (Scheme 1) by reduction of *N*-Boc-L-tryptophan followed by protection of the amine groups to give alcohol **4**. Subsequent alkylation of **4** using sodium hydride and 3,5-dimethylbenzyl bromide gave ether **5** accompanied by substantial amounts of the oxazolidinone **6**; no attempt was made to improve this synthesis because of the poor activity of the acetamide **8** (see below). Amide analogues **9** and **10** were made using conventional amide coupling reactions and are detailed in the Experimental Section. Synthesis of the equivalent ketones has been described in a preliminary communication[11] and is detailed in Scheme 2. *N*-Boc-L-tryptophan was activated using isobutyl chloroformate and coupled to *N*,*O*-dimethylhydroxylamine to give the Weinreb amide **11**. This was reacted at low temperature[12] with lithium dimethyl methylphosphonate to give the keto phosphonate **12** which could be purified by chromatography but was more conveniently, after aqueous workup, condensed with 3,5-bis(trifluoromethyl)benzaldehyde to give the unsaturated ketone **13** as yellow crystals in 47% yield over the two steps. Reduction of the olefin was preferentially carried out under hydrogenation conditions using Wilkinson's catalyst and was followed by removal of the Boc protecting group with ethereal HCl to give the amino ketone **15** in 96% yield from **13**. Using this sequence, 50 g batches of **15** (hydrochloride salt) could be prepared in 36% overall yield from *N*-Boc-L-tryptophan with ee 90% as measured by ^1H NMR of the diastereomeric camphanamide derivatives. The enantiomeric excess was routinely enhanced to give homochiral material (within the limits of detection) by precipitation of the salt from hot methanol on addition of water. Acylation of amine **15** was variously carried out by carbodiimide-, carbonyldiimidazole-, or mixed anhydride-mediated couplings with acids or by reaction with acyl chlorides to give a range of amide derivatives, **16**.

Biology

A stable CHO cell line expressing the human NK_1 receptor was used[13] to determine binding affinities of compounds prepared in this series with [^{125}I]Tyr8-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.[13] NK_1 receptor binding assays with a Gln165Ala mutant were performed as previously described[14] and results compared with binding affinities for the wild-type receptor carried out under the same conditions.

Substance P-induced plasma extravasation assays were performed in guinea pigs. Test compounds were administered by oral dosing 1 h before a challenge with

Scheme 2

^{a} Reagents and conditions: (a) *i*-BuOCOCl, Et_3N, DMF, then HNMeOMe, −15 °C; (b) $LiCH_2PO(OMe)_2$, THF, −78 °C; (c) 3,5-bis(trifluoromethyl)benzaldehyde, K_2CO_3, CH_3CN; (d) Wilkinson's catalyst, EtOAc, H_2, 40 psi; (e) Et_2O, HCl.

0.5 pmol of substance P injected into the dorsal 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.

Cardiovascular effects of CP-99,994, CP-96,345, and **16g,i** were measured in ferrets of either sex (approximate body weight, 1.5 kg). Anesthesia was induced with sodium pentobarbitone (40 mg/kg ip) and maintained with sodium barbitone (300 mg/kg ip). The trachea was cannulated, and the ferrets were then artificially ventilated with room air. The femoral artery and vein were cannulated for the recording of blood pressure and the administration of test compounds, respectively.

Results and Discussion

In an earlier paper, we described a series of novel, nonpeptide substance P receptor antagonists based on esters of L-tryptophan. Optimal NK_1 affinities within this class were achieved with benzyl esters substituted at both *meta*-positions with a lipophilic group, where the strongly electronegative trifluoromethyl was particularly preferred. Structural variations around the α-amino group generally had less of an influence on activity although acyl derivatives were superior to a basic amine at this position. In order to improve the *in vivo* activity of these compounds, we investigated two separate approaches toward replacing the ester group while attempting to maintain binding affinity. In the preceding paper we described analogues in which the ester and the α-acylamino group are incorporated into a single heterocyclic ring providing compounds with NK_1 activity somewhat lower (>7-fold) in affinity than the related esters.[15] In this paper we describe an acyclic approach to replacing the ester through relatively conservative modifications to the reference *N*-acetyl- and *N*-benzoyltryptophan esters **3** and **17a,b** (hNK_1 IC_{50} = 1.6, 67, and 22 nM, respectively).

(17a) R = CH_3
(17b) R = Ph

We have previously shown,[16] from binding studies on point mutants of the human NK_1 receptor, that the benzyl group of the tryptophan ester antagonists interacts with the same residue on the receptor protein (His265) as the benzyl group of a series of quinuclidine ether antagonists (**18**) developed in our laboratories.

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This is consistent with the preference in both series for 3,5-disubstitution on the aryl ring to achieve optimal binding affinity. We have also shown[18] that the bicyclic ring system of **18** may be dismantled to give acyclic diphenylalaninyl ethers (**19**) which retain good binding affinity; the *S*-enantiomer of **19** has an IC_{50} of 2.5 nM at the NK_1 receptor. Assuming that the benzyloxy

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groups of the three series of compounds represented by **3**, **18**, and **19** can be superimposed in a model of receptor binding, our initial assessment of the tryptophan esters was that the ether type oxygen, rather than the carbonyl, would correspond to the oxygen in compounds **18** and **19** and would be important for high-affinity binding. Surprisingly, the ether **8** turned out (Table 1) to be more than 10-fold lower in affinity than the corresponding ester **17a**. Although there is a significant difference in the activity of these two compounds, it is not possible to rule out that this is due to the increased conformational flexibility of **8** introducing a larger entropy factor into the binding process. Amide analogues of the esters were prepared and proved to be even less complementary to the receptor with affinities of both the secondary amide **9** and its *N*-methyl homologue **10** reduced more than 50-fold from the analogous ester **17b**.

Table 1. Human NK_1 Receptor Binding

compd	hNK_1 IC_{50} (nM)[a]	compd	hNK_1 IC_{50} (nM)[a]
17a	67 ± 10	**9**	1467 ± 450
17b	22 ± 3	**10**	1033 ± 330
8	767 ± 368		

[a] Displacement of ^{125}I-labeled substance P from the cloned human NK_1 receptor expressed in CHO cells. All binding data are reported as the mean ± SD for $n \geq 3$ determinations.

(9) R = H
(10) R = CH_3

In contrast to this, the *N*-acetylamino ketone **16a** had binding affinity (Table 2) within 2-fold of the corresponding ester **3**, suggesting that the ethereal ester oxygen plays no role in the binding of these compounds. The difference in activity of the amides, when compared with the esters and ketones, is quite striking and suggests either that the amides are unable to adopt the required binding conformation because of reduced freedom of bond rotation, or that the amide N-H or *N*-methyl adversely interacts with the receptor.[17] In the preceding paper[15] we described the activities of conformationally restricted hydantoin and diketopiperazine analogues of **10** which have binding affinities >100-fold weaker than the corresponding ester **3**. It is notable that two distinct *cis* and *trans* amide rotamers, in a ratio of 3:2, are observed in the DMSO-d_6 ^1H NMR spectrum for **10** at 300 K. The signals for these rotamers coalesce between

Table 2. Human NK1 Receptor Binding

Compound	R	Method of Preparation[a]	hNK_1 IC_{50} (nM)[b]
CP 96,345			0.45
CP 99,994			0.5
3			1.6
16a	$-CH_3$	A	3.1 ± 1.7
16b	$-CH_2NMe_2$	B	5.0 ± 3.6
16c	$-(CH_2)_2NMe_2$	B	1.8 ± 0.2
16d	$-(CH_2)_3NMe_2$	B	0.7 ± 0.2
16e	$-(CH_2)_4NMe_2$	A	0.6 ± 0.1
16f	[structure]	B	3.5 ± 0.7
16g	[structure]	C	0.5 ± 0.1
16h	[structure]	C	3.8 ± 0.8
16i	[structure]	D	0.9 ± 0.4
16j	$-(CH_2)_2N$ [structure]	D	0.6 ± 0.3
16k	$-(CH_2)_3N$ [structure]	E	0.3 ± 0.1
16l	$-(CH_2)_3N$ [structure]	E	0.17 ± 0.05
16m	$-(CH_2)_2-$ [structure]	E	0.47 ± 0.05
16n	$-(CH_2)_2-$ [structure]	D	0.37 ± 0.05

[a] Coupling of **15** with appropriate acid mediated by: A—see Experimental Section; B—isobutyl chloroformate; C—carbonyldiimidazole; D—acid chloride; E—water soluble carbodiimide. A detailed example for each of B−E is given in the Experimental Section. [b] Displacement of ^{125}I-labeled substance P from the cloned human NK_1 receptor expressed in CHO cells. All binding data are reported as the mean ± SD for $n \geq 3$ determinations.

350 and 360 K indicating a substantial barrier to rotation. As may be expected, the secondary amide **9** is a single entity (by NMR) at 298 K, presumably existing predominantly as the preferred *trans* amide.

The discovery that the hydrolytically labile ester in our screening lead could be effectively replaced by a ketone led us to investigate in more detail the structure−activity surrounding the α-acylamino substitutent in these compounds. In order to improve on the very low aqueous solubility of **16a**, a number of amine-containing derivatives were prepared. The *N,N*-dimethylglycinamide derivative **16b** was marginally lower in affinity than **16a**, while the aminopropionamide **16c** was 2-fold more active. Further homologation **(16d)** of the amine increased affinity an additional 4-fold over **16a**, while extending to the four-methylene spacing in **16e** gave no further benefit in binding. The aminoacyl side chain of these compounds was modified to investigate the effect of reducing conformational flexibility and lowering the pK_a of the basic group and to probe steric bulk

Table 3. Binding to Gln165Ala Mutant Receptor

compound	IC_{50} (nM)[a] hNK₁[b]	Q165A[c]	ratio[d]
CP-96,345	0.5	22	44
20	53	2100	40
3	3.3 ± 0.6	8.1 ± 2	2
16a	2.2 ± 0.5	10 ± 5	5
16g	0.4 ± 0.1	10 ± 6	25
16i	1.0 ± 0.2	8 ± 4	8
16f	2.0 ± 0.8	4 ± 1	2
16l	0.24 ± 0.07	1.4 ± 0.2	5

[a] Binding affinities at [b] wild-type and [c] Gln165Ala mutant receptors. A modified protocol[16] for these binding experiments accounts for small differences in wild-type data compared to Table 2. [d] Ratio of hNK_1/Q165A IC_{50}s.

tolerance at the NK_1 receptor. Within the series of compounds examined (Table 2), it was found that high-affinity binding was maintained with a variety of basic groups. The pK_a of these groups appeared to have little influence on binding when comparing pairs of compounds in which the amine nitrogen atoms can access the same region of the receptor. For example, the morpholine **16l** was as potent as pyrrolidine **16k**, while the weakly basic pyridine **16m** was just as active as its piperidine analogue **16n**. As with the dimethylamino compounds (**16b−e**), the position of the amine relative to the core of the molecule was a more important determinant in achieving the enhanced affinity seen with, for example, the morpholinylbutyramide **16l** (0.17 nM) compared to its acetamide analogue **16f** (3.5 nM).

Recent studies[14] carried out in our laboratories on mutants of the NK_1 receptor have shown that a residue, on the fourth transmembrane domain of the seven-transmembrane helical bundle that makes up this receptor protein, is critical for the binding of CP-96,-345 and quinuclidine ether substance P antagonists (e.g., **20**). Gln165 was shown to interact with the C3

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heteroatom, either nitrogen in CP-96,345 or oxygen in the ethers, most likely through a hydrogen-bonding interaction. Replacing Gln165 for alanine in the human receptor caused a 40−44-fold reduction in affinity for these compounds. In contrast, the binding of *N*-acetyltryptophan ester **3** to the Gln165Ala mutant was measured and found (Table 3) to be little changed from its affinity for the wild-type receptor, indicating that **3** does not rely on the side chain of residue 165 for its binding. Similarly, and as expected, the ketone analogue (**16a**) of **3** had affinity at the mutant receptor which was only 5-fold lower than at the wild-type receptor. When the higher affinity ketone ligands were tested, the consequence of this mutation was variable. All of the compounds assayed exhibited a reduction in affinity, but this varied from only 2-fold for **16f** to 25-fold for **16g**. The quinuclidinyl and pyridinyl compounds (**16i,g**) had the same affinity for the mutant receptor as the acetamide (**16a**), while the ligand with highest affinity for the wild-type receptor (**16l**) remained the most active at the mutant receptor. These data

Table 4. Inhibition of Plasma Extravasation

compound	ID_{50} (mg/kg)[a]	compound	ID_{50} (mg/kg)[a]
CP-99,994	1.6	**16g**	1.9
16c	~10	**16i**	1.8
16d	~4		

[a] Inhibition of substance P-induced dermal plasma extravasation in the guinea pig 1 h after oral dosing of test compound.

suggest that the ketone carbonyl in this series of compounds does not bind to the same residue as the exocyclic heteroatom in the quinuclidine antagonists and that Gln165 may contribute either directly or indirectly to the higher affinity binding of some of these ligands but is not essential to the activity of the series as a whole.

Compounds from this series with high receptor binding affinity (IC_{50} < 1 nM) were assayed for their ability to inhibit substance P-induced plasma extravasation in the guinea pig. In this model CP-99,994 was an effective inhibitor (Table 4) of extravasation with an ID_{50} of 1.6 mg/kg after oral dosing. Within the group of compounds tested, no direct correlation was found between *in vivo* activity and *in vitro* binding affinity, reflecting differences in bioavailability or pharmacokinetics depending on the nature of the amine-containing side chain. The 4-pyridylacetamide **16g** was equivalent in potency to CP-99,994 with an ID_{50} of 1.9 mg/kg. In order to establish that the activity of **16g** in this assay is specifically mediated through action at the NK_1 receptor, its effect on extravasation caused by either PAF or histamine was examined. No inhibition could be seen with this compound when tested against either of these two proinflammatory agents. The quinuclidine derivative **16i** (L-737,488) had marginally lower affinity than **16g** at NK_1 receptors, but oral activity for inhibition of plasma extravasation in the guinea pig was equivalent (ED_{50} = 1.8 mg/kg) to that of both **16g** and CP-99,994. Sustained activity was seen in the guinea pig with 72% inhibition of extravasation measured at 3 h after dosing with **16i** (3 mg/kg po). Although *in vivo* activities for **16i,g** were essentially the same, the higher pK_a of the quinuclidine resulted in greater aqueous solubility than for the pyridine (**16g**, <10 μg/mL at pH 7.4) with a maximum solubility of 2.2 mg/mL for **16i**.

Some of the effects seen with CP-96,345 in early evaluation of its activity have now been attributed[19] to its binding to the calcium channel and are also seen with its enantiomer (CP-96,344) which has similar calcium channel affinity but 100-fold lower binding affinity at the NK_1 receptor. Compound **16i** is more than 10-fold weaker (IC_{50} = 2.8 μM) than CP-96,345 in displacement of diltiazem from rabbit skeletal muscle, while **16g** is essentially inactive at the ion channel. The influence of these compounds on heart rate and blood pressure in the anesthetized ferret was evaluated to rule out cardiovascular effects in their ability to inhibit plasma extravasation (Tables 5 and 6). At rising cumulative doses up to 3 mg/kg (iv), there was essentially no change in heart rate with **16i** or **16g** relative to vehicle-matched controls. The initial bolus on injection of each compound caused a modest, transient decrease in blood pressure which returned to normal within 1 min. CP-99,994 had a similar profile to these compounds under the same conditions, but CP-96,345 caused a dose dependent and sustained (>15 min) decrease in heart rate and a dose dependent decrease in blood pressure reaching a sustained (>15 min duration) fall of 63% after the highest dose.

Table 5. Effect on Blood Pressure of Substance P Antagonists[a]

	dose (mg/kg)[b]				
compound	vehicle[c]	0.1	0.3	1.0	3.0
CP-96,345	−5 ± 4	−11 ± 5	−21 ± 7	−46 ± 4	−63 ± 4
CP-99,994	20 ± 9	10 ± 6	13 ± 9	12 ± 10	−31 ± 11
16g	−11 ± 3	NT	NT	−20 ± 3	−26 ± 2
16i	2 ± 4	−2 ± 3	−4 ± 4	−18 ± 16	−34 ± 10

[a] Maximum change in mean arterial blood pressure in the anesthetized ferret given as the mean ± SD of at least three determinations; changes were transient for all compounds except CP-96,345 (see text). [b] Given as an iv bolus. [c] Compounds were administered in distilled water (dosing volume, 1 mL/kg) except for **16g** which was dissolved in poly(ethylene glycol) (dosing volume, 0.5 mL/kg). The appropriate vehicle controls were used in each case.

Table 6. Effect on Heart Rate of Substance P Antagonists[a]

	dose (mg/kg)[b]				
compound	vehicle[c]	0.1	0.3	1.0	3.0
CP-96,345	0 ± 0	−7 ± 3	−13 ± 5	−21 ± 6	−65 ± 5
CP-99,994	−1 ± 2	−2 ± 1	−1 ± 2	−3 ± 3	−7 ± 4
16g	−7 ± 1	NT	NT	−11 ± 1	−7 ± 4
16i	3 ± 2	−3 ± 2	−2 ± 3	−1 ± 5	−1 ± 6

[a] Changes in heart rate in the anesthetized ferret given as the mean ± SD of at least three determinations. [b,c] See footnotes for Table 5.

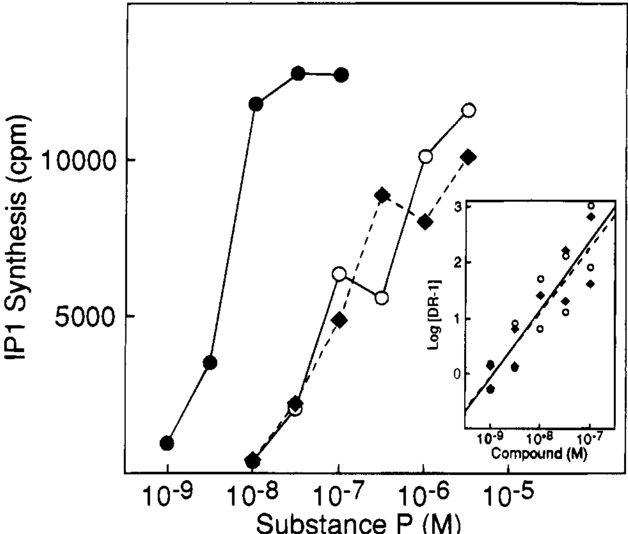

Figure 1. Inhibition of inositol monophosphate (IP1) synthesis in CHO cells by **16g** (○) and **16i** (◆), both at 30 nM, compared to control (●). Inset—Schild analysis of IP1 data.

The binding of **16i** to other members of the neurokinin family of receptors was measured, and it was found to be highly selective toward NK_1 with affinities at NK_2 and NK_3 greater than 1 μM (IC_{50}). The addition of increasing concentrations of either **16g** or **16i** resulted in an increase in the EC_{50} of substance P for stimulation of inositol phosphate synthesis in human NK_1/CHO cells without lowering the maximal response to the agonist (Figure 1). Schild analyses of these data were linear with slopes of 1.1 for both **16g,i** suggesting that, like the earlier[20] tryptophan ester **3**, these compounds function as competitive antagonists of substance P at the human receptor. The K_b values for these compounds in this system were 1.1 and 0.9 nM for **16g,i**, respectively.

Summary

The value of screening large established chemical sample collections for lead structures has been proven in a number of drug discovery programs. For the substance P receptor, the weakly active antagonist *N*-ethyltryptophan benzyl ester was recognized as a novel structural lead with potential for improvement to give compounds with higher affinity. Initial structure–activity relationship (SAR) studies retaining the ester group of this lead demonstrated that high-affinity ligands can be obtained by correct choice of substitution on the phenyl ring and the α-amino group. The second objective within this program, to improve *in vivo* activity, has been achieved by converting the ester to a ketone which provides compounds with affinity comparable to the equivalent esters. The synthesis described for the ketones allows for the large scale preparation of compounds with high enantiomeric purity. An exploration of acylamino substituents led to a further 10-fold increase in affinity, and incorporation of an amine into the side chain provided significant aqueous solubility. From the group of ketones tested *in vivo*, the quinuclidine derivative L-737,488 (**16i**) has been shown to have good oral activity in an animal model of inflammation, with no significant cardiovascular effects. The evaluation of compounds from this series in other models of disease states in which substance P is involved is in progress.

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 used in chromatography had bp 60–80 °C. A Hewlet-Packard 1090L instrument was used for HPLC analyses. Elemental analyses were determined by Butterworth Laboratories Ltd., Teddington, England.

1-[(3,5-Dimethylbenzyl)oxy]-2-acetamido-3-(3-indolyl)-propane (8). L-Tryptophan (10.2 g, 50 mmol) was cautiously added in portions to a stirred solution of $LiAlH_4$ in THF (1.0 M, 150 mL, 5 mmol). The reaction mixture was stirred for 72 h and then heated to reflux for 1 h. The reaction mixture was cooled and then the reaction quenched carefully with 2 N NaOH (150 mL). EtOAc (500 mL) was added, and the mixture was filtered through a pad of Celite. The organic phase was washed with water, dried, and evaporated to yield crude 2-amino-3-(3-indolyl)-1-propanol (4.6 g). The amino alcohol was dissolved in CH_3CN and treated with DMAP (2.95 g, 24.2 mmol) and Boc_2O (7.26 g, 33.3 mmol) at 20 °C. The reaction mixture was stirred for 2 h and the solvent evaporated. The residue was dissolved in MeOH (500 mL), KOH (1.3 g) was added, and the reaction mixture was stirred for 1 h before the solvent was removed and the residue partitioned between EtOAc and water. The organic phase was dried and evaporated and the residue purified by chromatography using EtOAc/petroleum ether (1:4) to yield **4** (3.8 g, 9.7 mmol, 40%). This alcohol was dissolved in DMF (10 mL) and THF (40 mL), treated with NaH (80% dispersion in oil, 0.43 g, 14.3 mmol), and stirred for 15 min before adding 3,5-dimethylbenzyl bromide (2.9 g, 14.6 mmol). The reaction mixture was stirred for 16 h before removing the solvent and partitioning between EtOAc and water. The organic phase was dried and evaporated to give a residue which was purified by chromatography on silica gel using EtOAc/petroleum ether (1:4). The resulting oil was dissolved in methanolic HCl and stirred for 16 h, and then the solvent was removed and the residue partitioned between EtOAc and aqueous K_2CO_3. The organic phase was dried, filtered, and evaporated, and the residue was purified by chromatography using CH_2Cl_2/MeOH (9:1) to yield an oil which was treated with ethereal oxalic acid to give **7** as a hygroscopic solid (0.150 g, 5%): ^{1}H NMR (DMSO-d_6) δ 11.04 (1H, s), 7.56 (1H, d, J = 8 Hz), 7.37 (1H, d, J = 7 Hz), 7.00 (1H, t, J = 7 Hz), 6.93 (2H, s), 6.90 (1H, s), 4.43 (1H, d, J = 12 Hz), 4.36 (1H, d, J = 12 Hz), 3.54–3.42 (3H, m), 3.09–2.96 (2H, m), 2.24 (6H, s); MS (CI$^+$) 309 [M + H]$^+$. Anal. ($C_{20}H_{24}N_2O{\cdot}(COOH)_2$) C, H, N.

Compound **7** (0.5 g, 1.45 mmol) was dissolved in dry pyridine (0.5 mL), and Ac_2O (0.5 mL) was added. The reaction mixture was stirred for 16 h, and then EtOAc (50 mL) was added. The solution was washed with 5 N HCl (50 mL), brine (50 mL), and water (50 mL). The organic phase was dried and filtered and the solvent removed *in vacuo* to yield an oil which was purified by chromatography using EtOAc/petroleum ether (3:2) to yield **8** as an amorphous solid (75 mg, 14%): ^{1}H NMR ($CDCl_3$) δ 8.05 (1H, s), 7.72 (1H, d, J = 8 Hz), 7.34 (1H, d, J = 8 Hz), 7.19 (1H, t, J = 7 Hz), 7.12 (1H, t, J = 7 Hz), 6.96 (4H, s), 5.85 (1H, d, J = 8 Hz), 4.44–4.36 (1H, m), 4.42 (2H, s), 3.48–3.38 (2H, m), 3.11–2.99 (2H, m), 2.33 (6H, s), 1.94 (3H, s); MS (CI$^+$) 351 [M + H]$^+$. Anal. ($C_{22}H_{26}N_2O_2{\cdot}0.2H_2O$) C, H, N.

***N*-(3,5-Dimethylbenzyl)-2-benzamido-3-(3-indolyl)propionamide (9).** *N*-α-Benzoyl-L-tryptophan (0.67 g) was dissolved in dry DMF (15 mL). The solution was cooled to 0 °C, and 1-hydroxybenzotriazole (0.3 g), followed by 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.433 g), was added. The reaction mixture was stirred for 0.5 h, and then 3,5-dimethylbenzylamine (0.3 g) was added and the reaction mixture stirred for 16 h. The reaction mixture was filtered, diluted with CH_2Cl_2 (500 mL), and washed with a saturated $NaHCO_3$ solution (100 mL), brine (100 mL), and water (100 mL). The separated organic phase was dried, filtered, and evaporated to yield an oil which was purified by chromatography using CH_2Cl_2/MeOH (98:2) to give the title compound as a white solid (0.23 g, 25%): mp 177–178 °C; ^{1}H NMR ($CDCl_3$) δ 7.75 (2H, d), 7.71 (1H, d) 7.50–7.36 (5H, m), 7.16–7.04 (4H, m), 6.64 (1H, s), 6.72 (2H, s), 4.96 (1H, q), 4.26 (2H, ABX), 3.37 (2H, ABX), 2.23 (6H, s). Anal. ($C_{27}H_{27}N_3O_2$) C, H, N.

***N*-(3,5-Dimethylbenzyl)-*N*-methyl-2-benzamido-3-(3-indolyl)propionamide (10).** *N*-Boc-(3,5-dimethylbenzyl)amine (0.95 g) was treated with NaH (0.16 g of a 60% dispersion in oil) in dry THF. The reaction mixture was stirred for 10 min before adding MeI (1 mL) and stirring for 16 h. The solvent was removed, and the residue was partitioned between EtOAc and water. The organic phase was dried, filtered, and evaporated to yield a white solid, which was dissolved in methanolic HCl and heated to reflux for 30 min. The solvent was removed by evaporation and the residue partitioned between 2 N NaOH and EtOAc. The organic phase was dried and evaporated to yield crude *N*-methyl-(3,5-dimethylbenzyl)-amine (0.28 g). Following the method described above for **9**, *N*-α-benzoyl-L-tryptophan and *N*-methyl-(3,5-dimethylbenzyl)-amine gave the title compound: mp 140–142 °C; ^{1}H NMR at 297 K showed two rotameric species in a ratio of approximately 3:2 (DMSO-d_6) δ 7.90 (1.2H, d), 7.84 (0.8H, d), 7.63 (0.6H, d), 7.53–7.23 (5H, m), 7.14 (0.4H, s), 7.08–6.97 (2H, m), 6.88–6.84 (1H, m), 6.77 (0.8H, s), 6.72 (1.2H, s), 5.22–5.15 (1H, m), 4.65 (0.4H, d), 4.53–4.57 (1.2H, m), 4.31 (0.4H, d), 3.29–3.14 (2H, m), 2.88 (1.8H, s), 2.78 (1.2H, s), 2.20 (3.6H, s), 2.17 (2.4H, s). Anal. ($C_{28}H_{29}N_3O_2$) C, H, N.

***N*-Methoxy-*N*-methyl-2-(*N*-Bocamino)-3-(3-indolyl)propionamide (11).** *N*-α-Boc-L-tryptophan (100 g, 0.32 mol) was dissolved in DMF (800 mL), and Et_3N (101 g, 1 mol) was added. The reaction mixture was cooled to −30 °C, and isobutyl chloroformate (42.5 mL, 0.32 mol) was added, maintaining the internal temperature below −20 °C. The reaction mixture was stirred for 15 min before adding MeONHMe·HCl (64 g, 0.65 mol) and then diluting the reaction mixture with CH_2Cl_2 (1 L), maintaining the internal temperature below 0 °C. The reaction mixture was stirred for 15 min, poured into EtOAc (3 L), and washed with 10% citric acid (1 L), water (3 × 1 L),

saturated $NaHCO_3$ (1 L), and water (1 L). The organic phase was dried, filtered, and evaporated until crystallization ensued. The suspension was diluted with petroleum ether, filtered, and dried to yield the title compound (90.4 g, 84%): mp 129–130 °C; ^{1}H NMR (DMSO-d_6) δ 10.80 (1H, s), 7.51 (1H, d, J = 7 Hz), 7.33 (1H, d, J = 7 Hz), 7.16 (1H, s), 7.08–6.97 (3H, m), 4.62–4.58 (1H, m), 3.72 (3H, s), 3.34 (3H, s), 3.02–2.81 (2H, m), 1.31 (9H, s); $[\alpha]_D$ −11.2° (c = 1, MeOH).

2-(*N*-Bocamino)-1-(3-indolyl)-4-(dimethylphosphono)-3-butanone (12). $MePO(OMe)_2$ (205 g, 1.65 mol) was dissolved in THF (800 mL), cooled to −70 °C, and then treated with n-BuLi (1.6 M in hexane, 900 mL, 1.44 mol), maintaining the internal temperature of the reaction mixture below −55 °C. The reaction mixture was stirred for 1 h before adding **11** (90 g, 0.27 mol). The reaction mixture was stirred at −70 °C for 30 min before the reaction was quenched with saturated NH_4Cl. The resulting mixture was extracted with EtOAc and the organic extract washed with water (5 × 500 mL), dried, and evaporated. The residue was purified by chromatography (eluting with EtOAc) to yield the title compound as an oil (69.0 g, 63%): ^{1}H NMR ($CDCl_3$) δ 10.84 (1H, s), 7.56 (1H, d, J = 7 Hz), 7.33 (1H, d, J = 7 Hz), 6.98 (1H, t, J = 7 Hz), 4.34–4.31 (1H, m), 3.63 (6H, d, J = 11 Hz), 3.39 (2H, d, J = 22 Hz), 3.19–3.11 (1H, m), 2.91–2.84 (1H, m); $[\alpha]_D$ −22.2° (c = 1, MeOH). Anal. ($C_{19}H_{27}N_2O_6P$) C, H, N.

5-[3,5-Bis(trifluoromethyl)phenyl]-2-(*N*-Bocamino)-1-(3-indolyl)-4-penten-3-one (13). A solution of **10** (69.0 g, 0.17 mol) in acetonitrile (600 mL) was stirred with K_2CO_3 (28 g, 203 mmol) and 3,5-bis(trifluoromethyl)benzaldehyde (55 g, 0.23 mol). The reaction mixture was stirred for 16 h, and then the mixture was filtered and the solvent evaporated. The yellow solid obtained was recrystallized from EtOAc/hexane to give the product as yellow crystals (77.6 g, 87%): mp 137–138 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (1H, s), 7.81 (1H, s), 7.72 (1H, d, J = 7 Hz), 7.53 (2H, s), 7.43 (1H, d, J = 18 Hz), 7.39 (1H, d, J = 7 Hz), 7.30–7.14 (2H, m), 6.98 (1H, d, J = 2 Hz), 6.54 (1H, d, J = 18 Hz), 5.43–5.41 (1H, m), 5.08–5.04 (1H, m), 3.41–3.36 (1H, m), 3.18–3.13 (1H, m), 3.36 (1H, m), 1.46 (9H, s); $[\alpha]_D$ +53.4° (c = 1, MeOH). Anal. ($C_{26}H_{24}F_6N_2O_3$) C, H, N.

5-[3,5-Bis(trifluoromethyl)phenyl]-2-(*N*-Bocamino)-1-(3-indolyl)-3-pentanone (14). Compound **13** (52.4 g, 0.1 mol) in EtOAc (500 mL) was shaken under an atmosphere of H_2 gas at 60 psi in the presence of $(Ph_3P)_3RhCl$ for 2 h. The reaction mixture was filtered and concentrated *in vacuo*, and then the residue was crystallized from petroleum ether (bp 60–80 °C) to give **14** (52.6 g, 100%): mp 138–140 °C; ^{1}H NMR ($CDCl_3$) δ 8.03 (1H, s), 7.88 (1H, s), 7.57 (1H, d, J = 7 Hz), 7.36 (1H, d, J = 7 Hz), 7.35 (2H, s), 7.22 (1H, t, J = 7 Hz, 7.14 (1H, t, J = 7 Hz), 6.89 (1H, s), 5.18 (1H, m), 4.58 (1H, m), 3.25–3.19 (1H, m), 3.11–3.05 (1H, m), 2.88–2.38 (4H, m), 1.42 (9H, s); $[\alpha]_D$ +5.6° (c = 1, MeOH). Anal. ($C_{26}H_{24}F_6N_2O_3$) C, H, N.

2-Amino-5-[3,5-bis(trifluoromethyl)phenyl]-1-(3-indolyl)-3-pentanone Hydrochloride (15). Compound **14** (52.6 g, 0.1 mol) was dissolved in ethereal HCl and left to stand for 16 h. The solvent was evaporated to give **15** (46.3 g, 100%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 11.13 (1H, s), 8.42 (2H, bs), 7.89 (1H, s), 7.82 (2H, s), 7.61 (1H, d, J = 7 Hz), 7.38 (1H, d, J = 7 Hz), 7.26 (1H, d, J = 1 Hz), 7.10 (1H, t, J = 7 Hz), 7.01 (1H, t, J = 7 Hz), 4.36 (1H, t, J = 7 Hz), 3.34–2.77 (6H, m); $[\alpha]_D$ +42.3° (c = 1, MeOH). Anal. ($C_{21}H_{18}F_6N_2O{\cdot}HCl$) C, H, N. Optical purity was assessed by preparation of amide derivatives with camphanic acid chloride and subsequent ^{1}H NMR analysis from which an enantiomeric excess of 92% was estimated. Optical purity of this material was enhanced by dissolving in boiling methanol to which water was added until precipitation occurred. The solid was filtered and dried to give **15** (typically, 85% recovery of enriched material was obtained): mp 194–196 °C; $[\alpha]_D$ +46.0° (c = 1, MeOH) which was enantiomerically pure within the limits of detection.

Compounds **16** were prepared by various coupling methods as indicated in Table 2. Examples of each type of preparation are given below. Compounds not specifically detailed may be prepared by analogy with these methods.

Method A: 2-Acetamido-5-[3,5-bis(trifluoromethyl)phenyl]-1-(3-indolyl)-3-pentanone (16a). Compound **15** (0.2 g, 0.43 mmol) was treated with Ac_2O/pyridine in the same manner as **7** to yield the title compound as a white solid (190 mg, 91%): mp 50–53 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 10.84 (1H, s), 8.24 (1H, d), 7.87 (1H, s), 7.82 (2H, s), 7.49 (1H, d), 7.33 (1H, d, J = 8 Hz), 7.13 (1H, s), 7.06 (1H, dd, J = 7 Hz), 6.98 (1H, t, J = 7 Hz), 4.52 (1H, dd), 3.07 (1H, dd), 2.68–2.93 (5H, m), 1.79 (3H, s); MS (CI$^+$) 488 $[M + NH_4]^+$. Anal. ($C_{23}H_{20}F_6N_2O_2$) C, H, N.

Method B: 5-[3,5-Bis(trifluoromethyl)phenyl]-1-(3-indolyl)-2-((*N*-morpholinyl)acetamido]-3-pentanone Hydrochloride (16f). Potassium 2-(*N*-morpholinyl)acetate (0.2 g, 1.1 mmol) and Et_3N (0.1 g, 1.08 mmol) were dissolved in dry DMF and cooled to −30 °C before adding isobutyl chloroformate (0.147 g, 1.08 mmol). The reaction mixture was stirred for 20 min before adding **15** (0.5 g, 1.06 mmol). The reaction mixture was stirred for 3 h, poured into water, and then partitioned between EtOAc and water. The organic phase was washed with water (100 mL), saturated $NaHCO_3$ (100 mL), and water (100 mL). The organic extract was dried, filtered, and evaporated and the residue purified by chromatography using EtOAc. The resulting oil was treated with ethereal HCl to yield **16f** (291 mg, 49%): mp 83–86 °C; ^{1}H NMR (DMSO-d_6 + TFA) δ 7.88 (3H, s), 7.56 (1H, d, J = 14 Hz), 7.35 (1H, d, J = 14 Hz), 7.17 (1H, d, J = 2 Hz), 7.08 (1H, t, J = 14 Hz), 7.00 (1H, t, J = 14 Hz), 4.71–4.77 (1H, m), 3.65–4.02 (6H, m), 2.96–3.34 (10H, m); MS (CI$^+$) 556 $[M + H]^+$. Anal. ($C_{27}H_{27}F_6N_3O_3{\cdot}HCl{\cdot}0.5H_2O$) C, H, N.

Method C: 5-[3,5-Bis(trifluoromethyl)phenyl]-1-(3-indolyl)-2-[(4-pyridyl)acetamido]-3-pentanone Hydrochloride (16g). 4-Pyridylacetic acid (210 mg, 1.2 mmol) was suspended in a solution of Et_3N (234 mg, 2.3 mmol) in DMF (4 mL) and CH_2Cl_2 (5 mL). Carbonyldiimidazole (196 mg, 1.2 mmol) was added to the suspension, and the mixture was stirred for 0.5 h before adding **15** (0.5 g, 1.06 mmol). The reaction mixture was stirred for 12 h, poured into EtOAc, and washed with water. The organic layer was dried, evaporated, and purified by chromatography using CH_2Cl_2/MeOH (98:2). The oil obtained was treated with ethereal HCl and crystallized from EtOH/EtOAc/Et_2O to yield **16g** (449 mg, 77%): mp 88–90 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 10.89 (1H, s), 8.77 (1H, d), 8.68 (2H, d, J = 6.5 Hz), 7.87 (1H, s), 7.83 (2H, s), 7.65 (2H, d, J = 6.5 Hz), 7.50 (1H, d, J = 8 Hz), 7.34 (1H, d, J = 8 Hz), 7.13 (1H, s), 7.06 (1H, t, J = 8 Hz), 7.96 (1H, t, J = 8 Hz), 4.58–4.66 (1H, m), 3.15 (1H, dd, J = 14.6 and 5.7 Hz), 2.75–2.98 (7H, m); MS (CI$^+$) 548 $[M + H]^+$; $[\alpha]_D$ +4.9° (c = 1, MeOH). Enantiomeric purity was determined by HPLC on a ChromTech CHIRAL-AGP column (eluent, 30% MeOH in 10 mM K_2HPO_4 at pH 7; flow rate, 1 mL/min; UV detection λ = 210 nm) giving an enantiomeric excess of 98.6%. Anal. ($C_{28}H_{23}F_6N_3O_2{\cdot}HCl{\cdot}H_2O$) C, H, N.

5-[3,5-Bis(trifluoromethyl)phenyl]-2-[5-(*N,N*-dimethylamino)pentanamido]-1-(3-indolyl)-3-pentanone (16e). A solution of **15** (1.1 g, 2.4 mmol) in CH_2Cl_2 (50 mL) was treated with chlorovaleryl chloride (0.52 mL, 4 mmol) and Et_3N (0.64 mL, 4.6 mmol) for 16 h. The reaction mixture was diluted with CH_2Cl_2, washed with dilute HCl and aqueous $NaHCO_3$, dried, and concentrated to give an oil. To a solution containing the foregoing oil in EtOH (5 mL) were added Me_2NH (5 mL of a 33% solution in EtOH) and KI (50 mg). After stirring for 4 days the mixture was partitioned between EtOAc and water. The EtOAc solution was separated, dried, and concentrated and the residue purified by chromatography eluting with EtOAc/MeOH (95:5) to give **16e** (102 mg, 8%): mp 140 °C; ^{1}H NMR ($CDCl_3$) δ 8.30 (1H, s), 7.67 (1H, s), 7.57 (1H, d, J = 8 Hz), 7.40 (2H, s), 7.39 (1H, d, J = 8 Hz), 7.21 (1H, t, J = 8 Hz), 7.13 (1H, t, J = 8 Hz), 6.91 (1H, d, J = 2 Hz), 6.40 (1H, d, J = 7 Hz), 4.87 (1H, q, J = 7 Hz), 3.20 (1H, dd, J = 6.5 and 14.5 Hz), 3.12 (1H, dd, J = 7.5 and 14.5 Hz), 2.67–2.09 (4H, m), 2.38–2.53 (2H, m), 2.32 (6H, s), 2.15–2.28 (2H, m), 1.50–1.67 (4H, m); MS (CI$^+$) 556 $[M + H]^+$. Anal. ($C_{28}H_{31}F_6N_3O{\cdot}0.5H_2O$) C, H, N.

Method D: 5-[3,5-Bis(trifluoromethyl)phenyl]-1-(3-indolyl)-2-(quinuclidine-4-carboxamido)-3-pentanone Hydrochloride (16i). Quinuclidine-4-carboxylic acid (*Helv. Chim Acta.* **1974**, *57*, 2332) (1.12 g, 5.8 mmol) was heated with thionyl chloride (5 mL) for 2 h. The solvent was removed and

the residue azeotroped with toluene (3 × 10 mL) and then dissolved in CH_2Cl_2 (10 mL). Compound **15** (2.25 g, 4.7 mmol) was dissolved in a mixture of CH_2Cl_2 (10 mL) and Et_3N (650 mg, 6.5 mmol) and added to the above solution. The reaction mixture was stirred for 1 h, poured into EtOAc, and washed with K_2CO_3 solution. The organic solution was dried, evaporated, and purified by chromatography on alumina using CH_2Cl_2/MeOH (99:1). The oil obtained was treated with ethereal HCl and crystallized from MeOH/H_2O; when a small quantity of crystals had formed, the mixture was filtered and the remaining solution evaporated to give **16i** (485 mg, 53%): mp 183–184 °C; ^1H NMR (DMSO-d_6) δ 8.06 (1H, d, J = 7.2 Hz), 7.88 (1H, s), 7.85 (2H, s), 7.51 (1H, d, J = 7.9 Hz), 7.33 (1H, d, J = 7.9 Hz), 7.12 (1H, s), 7.06 (1H, t, J = 7.9 Hz), 6.79 (1H, t, J = 7.9 Hz), 4.50–4.56 (1H, m), 3.12–3.24 (7H, m), 2.74–3.02 (5H, m), 1.83–1.88 (6H, m); $[\alpha]_D$ −1.2° (c = 2.5, MeOH). Anal. ($C_{29}H_{29}F_6N_3O_2$·HCl·H_2O) C, H, N. The enantiomeric purity was determined by HPLC of the free base on a Chiracel OD column (eluent, 30% EtOH in hexane; flow rate, 2 mL/min; UV detection, λ = 210 nm) giving an enantiomeric excess of 97.8%. Solubility in water was measured by dissolving the hydrochloride salt to saturation in deionized water. The solution was filtered through an Anotop 0.2 μm filter to give a clear solution (final pH 8.4) which was assayed by HPLC on a Spherisorb C18 column (eluent, 70% CH_3CN in 50 mM KH_2PO_4/0.1% Et_3N, pH 3, buffer; flow rate, 1 mL/min; UV detection, λ = 254 nm) and compared to a standard solution dissolved in CH_3CN. The concentration of **16i** was 2.2 mg/mL.

Method E: 5-[3,5-Bis(trifluoromethyl)phenyl]-2-[4-(1-pyrrolidinyl)butyramido]-1-(3-indolyl)-3-pentanone (16k). 4-Pyrrolidinylbutanoic acid hydrochloride (0.54 g, 2.8 mmol) in DMF (6 mL) was stirred with 1-[3-(dimethylamino)propyl]-3-carbodiimide hydrochloride (0.38 g, 2.0 mmol) for 1 h. CH_2Cl_2 (6 mL) and Et_3N (0.39 mL, 2.0 mmol) were added, the reaction mixture was stirred for 0.25 h, and **15** (0.46g, 1 mmol) was added. The reaction mixture was stirred for 2 h, poured into EtOAc (50 mL), washed with brine, dried, and concentrated to yield a solid which was recrystallized from Et_2O/petroleum ether to give **16k** (267 mg, 47%): mp 99–100 °C; ^1H NMR (DMSO-d_6) δ 10.82 (1H, s), 8.20 (1H, d, J = 6 Hz), 7.86 (1H, s), 7.83 (2H, s), 7.49 (1H, d, J = 8 Hz), 7.32 (1H, d, J = 8 Hz), 7.12 (1H, d, J = 2 Hz), 7.06 (1H, t, J = 8 Hz), 6.97 (1H, t, J = 8 Hz), 4.47–4.53 (1H, m), 3.09 (1H, dd, J = 6 and 15 Hz), 2.63–2.94 (5H, m), 2.05–2.27 (8H, m), 1.54–1.62 (6H, m); MS (CI$^+$) 568 $[M + H]^+$. Anal. ($C_{29}H_{31}F_6N_3O_2$) C, H, N.

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