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A flexible approach to the design of new potent substance P receptor ligands

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Abstract

The development of small-molecule antagonists of the substance-P-preferring tachykinin NK_1 receptor offers an excellent opportunity to exploit these molecules as novel therapeutic agents in diverse pathologies such as depression, emesis or asthma. GR71251 has previously been identified as a potent and selective substance-P-receptor antagonist. We have therefore undertaken the synthesis of new pseudopeptidic analogues based on the C-terminal sequence of GR71251. The evaluation of binding affinities toward NK_1 and NK_2 receptors has enabled us to propose new selective NK_1 ligands with high affinity. Structure–activity relationships showed that the Trp-OBzl$(CF_3)_2$ moiety is essential for NK_1 affinity and that the introduction of building units such as spirolactam, lactam or proline, leading to a constrained peptide, increased selectivity for NK_1 receptors. These compounds constitute a useful starting point for new substance P antagonists and represent an attractive lead series for further studies on the design of specific NK_1 antagonists.

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Introduction

Since the discovery of substance P more than 60 years ago (Von Euler & Gaddum 1931), the pharmacology of this neurotransmitter has been studied in great detail. Substance P, an undecapeptide with the sequence Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (Chang et al 1971), is a member of the tachykinin family which also includes neurokinin A (NKA) and neurokinin B (NKB), with the common C-terminal sequence Phe-xxx-Gly-Leu-Met-NH_2. Substance P mainly mediates its physiological effects by binding to specific NK_1 receptors whereas NKA and NKB exert their activities via NK_2 and NK_3 receptors, respectively. It has been established that substance P plays a key role in a wide range of biological processes (Maggi et al 1993; Mills 1997) and that it is involved in the transmission of pain signals, in the modulation of CNS disorders such as depression (Kramer et al 1998; Wahlestedt 1998) and in inflammatory processes such as migraine headaches (Beattie et al 1995), rheumatoid arthritis (Levine et al 1984) and asthma (Naline et al 1996). Consequently, there is a considerable interest in the action of this neurotransmitter.

Design

When we became interested in the conception of NK_1 antagonists, Ward et al (1990) had already used the incorporation of a bicyclic conformational constraint into the C-terminal sequence of substance P (Figure 1), which culminated in a competitive antagonist, GR71251, possessing high affinity ($pK_B = 7.7$) and selectivity for NK_1 receptors. This constraint excluded receptor-activating conformations but admitted the expression of residual antagonistic affinity.

In our conception of new NK_1 antagonists, we postulate that the rigidification of the substance P skeleton in GR71251 by spirolactam moiety may induce the displacement of the C-terminal substance P sequence to another binding site and precisely to the NK_1 antagonist site described (Cascieri et al 1994).

Moreover, Ward et al (1990) noted that replacement of Met^{11}-NH_2 of substance P by Trp-NH_2 led to an increase in the antagonistic activity of GR71251 that was not brought about by any modification in the C-terminal substance P sequence. So, we hypothesised that the C-terminal sequence of GR71251 was essential for any antagonist activity and had a direct influence on the recognition of NK_1 receptors. Furthermore, structure–activity studies on the site-directed mutagenesis of NK_1 receptors (McLeod et al 1993, 1994; Cascieri et al 1994; Millet et al 1999a, 1999b, 1999c) have shown the importance of the bis(trifluoromethyl)benzyl group for potent and selective NK_1 recognition, due to aromatic interactions with His^{265} of the NK_1 antagonist binding site. These features prompted us to design two spirolactam derivatives (Figure 1): compound **1a** which contains -Trp-NH_2 as amino acid and mimics the C-terminal sequence of GR71251 and **2a,** a spirolactam derivative which contains -Trp-OBzl$(CF_3)_2$ in its C-terminal sequence. In addition, we have described the synthesis of compounds **3** and **4** which include a Pro or lactam building unit in their N-terminal sequence and represent a simplified construction of spirolactam **2a**.

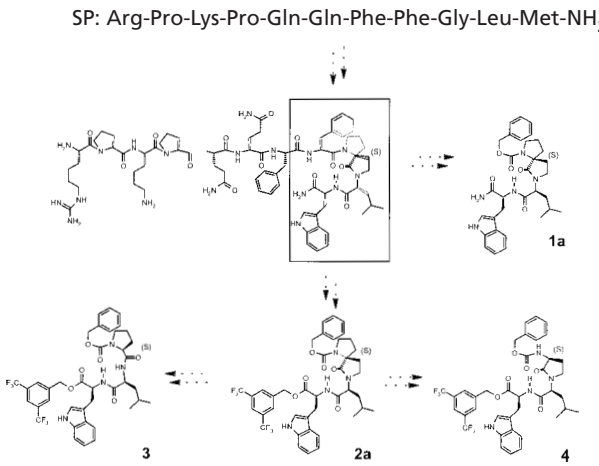

Figure 1 Drug design of spirolactam (**1a**, **2a**), proline (**3**) and lactam (**4**) derivatives.

Materials and Methods

Chemistry

Melting points were determined on a Büchi 535 capillary melting point apparatus and are uncorrected. Analytical thin-layer chromatography was performed on precoated Kieselgel $60F_{254}$ plates (Merck). The spots were located by UV (254 nm and 366 nm); Rf values are given for guidance. Column chromatographies were performed on silica gel 60 230–400 Mesh (Merck). IR spectra were determined in potassium bromide pellets with a Perkin Elmer 1310 spectrophotometer; absorbances are reported in ν (cm^{-1}). ^1H NMR spectra were recorded on a Bruker AC 300 spectrometer (300 MHz) using tetramethylsilane as an internal standard. Chemical shifts were expressed in δ units (ppm) and the splitting patterns were designated as follows: s, singlet; bs, broad singlet; t, triplet; d, doublet; dd, doublet of doublets; m, multiplet; bm, broad multiplet. Mass spectra (MS) were recorded on a quadripolar Finnigan Mat SSQ 710 instrument in the electron impact (EI) or chemical ionization (CI) mode. HPLC analyses were performed on a Hewlett-Packard 1090 liquid chromatograph, using a Licrospher 60 RP-select B C8, 5 μm 250×4.6 mm column (inverse phase) to estimate the purity of the final products tested. Elution was performed using solution A (80% water, 5% PIC B-8 low UV Reagent (Waters Part No WAT084283), 15% methanol) and solution B (10% water, 5% PIC B-8, 85% methanol). In the isocratic mode, percentages of solution A and B were noted. Elemental analyses for C, H, N, were performed by the Service Central d'Analyses (CNRS, Vernaison, France) and were within 0.4% of theory.

tert-*Butyl* N-*(benzyloxycarbonyl-2(*RS*)-allylprolinate (**5**)*

A solution of Cbz-DL-Pro-OtBu (10.0 g, 32.0 mmol, 1 equiv.) in dry tetrahydrofuran (THF) was cooled to −78°C (nitrogen). A THF solution of LDA (lithium diisopropylamide) (2 M, 19.7 mL, 39.3 mmol, 1.2 equiv.) was added drop-wise. After 20 min, allyl bromide (3.4 mL, 39.3 mmol, 1.2 equiv.) was added slowly. The solution was stirred at −78°C for 4 h, then allowed to warm to room temperature. The reaction was partitioned between CH_2Cl_2 and H_2O. The organic layer

was washed, dried and concentrated to give a yellow oil which was chromatographed on a flash column (5 × 40 cm) using hexane–ethyl acetate (95:5) as the eluting solvent. The product was isolated as a colourless oil. Yield, 70% (7.91 g). TLC: Rf (hexane–EtOAc, 95:5) = 0.25. IR (KBr) cm^{-1}: 1710 (C═O), 1685 (C═O). ^1H NMR ($CDCl_3$) δ: 1.34 (s, 4.5H), 1.39 (s, 4.5H), 1.79–1.91 (m, 2H), 2.02–2.11 (m, 2H), 2.51–2.63 (m, 1H), 2.89 (dd, 0.5H, $J = 6.4$ Hz, $J' = 14.7$ Hz), 3.09 (dd, 0.5H, $J = 6.4$ Hz, $J' = 14.7$ Hz), 3.41–3.47 (m, 1H), 3.63–3.72 (m, 1H), 4.99–5.23 (m, 4H), 5.63–5.77 (m, 1H), 7.28–7.38 (m, 5H). MS (CI) *m/z*: 346 (MH^+), 288 (M^+-$C(CH_3)_3$), 254 (M^+-CH_2-Ph), 245 (MH^+-$COOC(CH_3)_3$).

tert-*Butyl* N-*(benzyloxycarbonyl-2(*RS*)-(formylmethyl)prolinate (**6**)*

OsO_4 (250 mg) was added to a solution of **5** (5.93 g, 17.2 mmol, 1 equiv.) in THF–H_2O (4:1, 200 mL) (nitrogen). After 5 min, $NaIO_4$ (8.90 g, 40.8 mmol, 2.4 equiv.) was added in three batches over a 30-min period. After stirring for 4 h, the reaction was partitioned between Et_2O (100 mL) and H_2O (60 mL). The aqueous layer was extracted with Et_2O (3 × 50 mL). The combined Et_2O layers were washed, dried ($MgSO_4$) and concentrated. The tan oil obtained was chromatographed on a flash column (5 × 40 cm) using hexane–EtOAc (7:3) as the eluting solvent. Product **6** was isolated as a colourless oil. Yield, 80% (4.77 g). TLC: Rf (hexane–EtOAc, 7:3) = 0.45. IR (KBr) cm^{-1}: 1720 (C═O), 1700 (C═O). ^1H NMR ($CDCl_3$) δ: 1.26 (s, 3H), 1.33 (s, 6H), 1.78–1.96 (m, 2H), 2.10–2.22 (m, 2H), 2.66 (d, 0.33H, $J = 15.5$ Hz), 2.84 (d, 0.66H, $J = 14.8$ Hz), 3.03 (d, 1H, $J = 15.3$ Hz), 3.43–3.66 (m, 2H), 4.98 (d, 0.66H, $J = 12.5$ Hz), 5.01 (d, 0.33H, $J = 12.2$ Hz), 5.11 (d, 0.66H, $J = 12.5$ Hz), 5.14 (d, 0.33H, $J = 12.0$ Hz), 7.27–7.29 (m, 5H), 9.43 (s, 0.33H), 9.63 (s, 0.66H). MS (CI) *m/z*: 348 (MH^+), 290 (M^+-CH_2Ph), 247 (MH^+-$COOC(CH_3)_3$).

tert-*Butyl* N-*(benzyloxycarbonyl-2(*RS*)-(1-(*N*-((methoxycarbonyl)isopentyl)amino)ethyl)prolinate (**7**)*

Aldehyde **6** (4.00 g, 11.5 mmol, 1 equiv.) and NEt_3 (6.3 mL, 46.0 mmol, 4 equiv.) in MeOH (50 mL) were added to a mixture of H-Leu-OMe·HCl (8.36 g, 46.0 mmol, 4 equiv.) and 3-Å molecular sieves in MeOH (50 mL) (nitrogen). $NaCNBH_3$ (795 mg, 12.7 mmol, 1.1 equiv.) was added in one batch. The mixture was stirred for 24 h. The sieves were filtered off and the solvent was removed in-vacuo. The residue was chromatographed on a flash column (5 × 40 cm) using Et_2O as the eluting solvent to give **7** as a yellow oil. Yield, 80% (4.39 g). TLC: Rf (Et_2O) = 0.80. IR (KBr) cm^{-1}: 1710 (C═O), 1700 (C═O), 1685 (C═O). ^1H NMR (DMSO-d_6) δ: 0.85–0.96 (m, 6H), 1.30 (bs, 11H), 1.51–2.22 (m, 8H), 2.41–2.62 (m, 2H), 3.01–3.64 (m, 2H), 3.65 (s, 3H), 4.45–4.61 (m, 1H), 4.95–5.05 (m, 2H), 7.40 (s, 5H). MS (CI) *m/z*: 477 (MH^+), 385 (M^+-CH_2Ph).

*5(*RS*)-1-Benzyloxycarbonyl-1,7-diaza-7-(4(*S*)-methoxycarbonyl)-6-oxospiro[4,4]nonane (**8**)*

Trifluoroacetic acid (20 mL) was added to a cooled (ice bath) solution of **7** (3.80 g, 7.93 mmol, 1 equiv.) in CH_2Cl_2 (50 mL). The solution was stirred for 24 h then concentrated under reduced pressure. NEt_3 (2.85 mL, 19.8 mmol, 2.5 equiv.) and $CHCl_3$ (50 mL) were added and the solution was heated at reflux for 24 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2. The solution was washed successively with saturated aqueous $NaHCO_3$ solution, HCl (1 N) and H_2O. The organic phases were dried over $MgSO_4$ and chromatographed on a flash column (5 × 40 cm) using hexane–EtOAc (45:55) as the eluting solvent to give **8** as colourless crystals. Yield, 65% (2.08 g). mp 77–81°C. TLC: Rf (hexane–EtOAc, 4:6) = 0.35. IR (KBr) cm^{-1}: 1740 (C═O), 1700 (C═O), 1680 (C═O), ^1H NMR (DMSO-d_6) δ : 0.67–0.93 (m, 6H), 1.40–2.03 (m, 9H), 3.14–3.47 (m, 4H), 3.50–3.65 (m, 3H), 4.51–4.69 (m, 1H), 4.79 (d, 0.5H, $J = 13.0$ Hz), 4.90 (d, 0.5 H, $J = 13.0$ Hz), 5.10 (d, 0.5H, $J = 13.1$ Hz), 5.19 (d, 0.5H, $J = 13.1$ Hz), 7.25–7.40 (m, 5H). MS (CI) m/z: 403 (MH^+), 301 (M^+-$COOC(CH_3)_3$), 267 (M-CO_2CH_2Ph). Anal. Calcd for $C_{22}H_{30}N_2O_5$: C, 65.65; H, 7.51; N, 6.96. Found: C, 66.01; H, 7.52; N, 7.06.

*5(*RS*)-1-Benzyloxycarbonyl-1,7-diaza-7-((4(*S*)-carboxy)isopentyl)-6-oxospiro[4,4]nonane (**9**)*

NaOH solution (1 N, 10 mL) was added to a solution of ester **8** (1.0 g, 2.48 mmol, 1 equiv.) in MeOH (15 mL). The mixture was stirred for 20 min, and the organic solvent was evaporated. The solution was partitioned between CH_2Cl_2 and HCl (1 N). The organic phases were dried over $MgSO_4$. The oil obtained was chromatographed on a flash column (5 × 40 cm) using MeOH–CH_2Cl_2 (1:9) as the eluting solvent to give **9** as a white solid. Yield, 90% (868 mg). mp 78–90°C. TLC: Rf (toluene–acetic acid–acetone, 6:2:2) = 0.55. IR (KBr) cm^{-1}: 1700 (C═O), 1680 (C═O), 1650 (C═O). ^1H NMR (DMSO-d_6) δ: 0.65–0.91 (m, 6H), 1.40–2.05 (m, 9H), 3.15–3.45 (m, 4H), 4.50–4.71 (m, 1H), 4.71–4.90 (m, 1H), 5.10–5.20 (m, 1H), 7.25–7.40 (m, 5H), 12.40 (bs, 1H). MS (CI) *m/z*: 389 (MH^+), 387 (M^+ -H). Anal. Calcd for $C_{21}H_{28}N_2O_5$: C, 64.93; H, 7.27; N, 7.21. Found: C, 64.98; H, 7.44; N, 7.14.

(2S)-*(((1-Benzyloxycarbonyl)-1,7-diaza-(5*S *and 5*R*)-6-oxospiro[4,4]nonanyl)-4-methyl)pentanoyl (*S*)-tryptophan amides (**1a**) and (**1b**)*

H-Trp-NH_2 (543 mg, 2.27 mmol, 1.1 equiv.), PyBOP (benzotnazole-1-yltrispyrrolidinophosphonium hexafluorophosphate) (1.18 g, 2.27 mmol, 1.1 equiv.) and DIPEA (*N*,*N*-diisopropylethylamine) (0.9 mL, 5.15 mmol, 2.5 equiv.) were added to a solution of acid **10** (800 mg, 2.06 mmol, 1 equiv.) in 50 mL of CH_2Cl_2. The reaction was cooled in an ice bath and stirred for 48 h. The solution was partitioned between CH_2Cl_2 and HCl (0.5 N). The organic phases were dried ($MgSO_4$). The oil obtained was chromatographed on a flash column (5 × 40 cm) using isopropanol–EtOAc (5:95) as the eluting solvent to give the diastereoisomers **1a** and **1b** as white solids.

*(5*S*)-Diastereoisomer (**1a**)*. Yield, 40 % (472 mg). mp 98–99°C. TLC: Rf (CH_2Cl_2–MeOH, 95:5) = 0.3. IR (KBr) cm^{-1}: 3300 (NH), 1720 (C═O), 1685 (C═O), 1650 (C═O). ^{1}H NMR (DMSO-d_6) δ: 0.68–0.89 (m, 6H), 1.23–1.87 (m, 8H), 2.14–2.30 (m, 1H), 2.86–3.20 (m, 4H), 3.34–3.66 (m, 2H), 4.40–4.53 (m, 2H), 4.83 (d, 1H, J = 13.1 Hz), 5.13 (d, 1H, J = 12.9 Hz), 6.93–7.07 (m, 4H), 7.23–7.40 (m, 5H), 7.57 (d, 1H, J = 2.8 Hz), 7.81 (bs, 1H), 10.84 (s, 1H). HPLC: (isocratic 20 % A, 80 % B), t_R = 5.70 min. MS (CI) m/z: 575 (MH^+), 557 (M^+ -NH_3).

*(5*R*)-Diastereoisomer (**1b**)*. Yield, 40 % (472 mg). mp 104–106°C. TLC: Rf (CH_2Cl_2–MeOH, 95:5) = 0.3. IR (KBr) cm^{-1}: 3300 (NH), 1720 (C═O), 1685 (C═O), 1650 (C═O). ^{1}H NMR (DMSO-d_6) δ: 0.77–0.96 (m, 6H), 1.24–1.40 (m, 2H), 1.76–1.95 (m, 6H), 2.07–2.11 (m, 1H), 2.94–3.21 (m, 4H), 3.33–3.61 (m, 2H), 4.50–4.58 (m, 2H), 5.08 (d, 1H, J = 12.6 Hz), 5.15 (d, 1H, J = 12.6 Hz), 6.89–7.50 (m, 10H), 7.50–7.52 (m, 1H), 10.74 (s, 0.33H), 10.80 (s, 0.66H). HPLC : (isocratic 20 % A, 80 % B), t_R = 5.46 min. MS (CI) m/z: 575 (MH^+), 557 (M^+ -NH_3).

*(2*S*)-(((1-Benzyloxycarbonyl)-1,7-diaza-(5*S *and 5*R*)-6-oxospiro[4,4]nonanyl)-4-methyl)pentanoyl) (*S*)-tryptophan 3,5-bis(trifluoromethyl)benzyl esters (**2a**) and (**2b**)*

H-Trp-OBzl$(CF_3)_2$·HCl (793 mg, 1.70 mmol, 1.1 equiv.), PyBOP (888 mg, 1.70 mmol, 1.1 equiv.) and DIPEA (0.7 mL, 3.86 mmol, 2.5 equiv.) were added to a solution of acid **10** (600 mg, 1.54 mmol, 1 equiv.) in 50 mL of CH_2Cl_2. The reaction was cooled in an ice bath and stirred for 48 h. The solution was partitioned between CH_2Cl_2 and HCl (0.5 N). The organic phases were dried ($MgSO_4$). The oil obtained was chromatographed on a flash column (5 × 40 cm) using hexane–EtOAc (45:55) as the eluting solvent to give the diastereoisomers **2a** and **2b** as white solids.

*(5S)-Diastereoisomer (**2a**)*. Yield, 40 % (494 mg). mp 69–71°C. TLC: Rf(hexane–EtOAc,5:5) = 0.3. IR (KBr) cm^{-1}: 1720 (C═O), 1685 (C═O), 1650 (C═O). ^{1}H NMR (DMSO-d_6) δ: 0.61–0.68 (m, 6H), 1.23–1.84 (m, 8H), 2.10–2.30 (m, 1H), 3.08–3.47 (m, 6H), 4.54–4.56 (m, 2H), 4.81 (d, 1H, J = 13.1 Hz), 5.14 (d, 1H, J = 13.1 Hz), 5.16 (dd, 1H, J = 13.2 Hz, J' = 4.5 Hz), 5.25 (dd, 1H, J = 13.3 Hz, J' = 5.0 Hz), 6.96 (t, 1H, J = 7.2 Hz), 7.06 (t, 1H, J = 7.4 Hz), 7.16 (s, 1H), 7.26 (d, 1H, J = 6.0 Hz), 7.27–7.43 (m, 5H) 7.47 (d, 1H, J = 7.7 Hz), 7.98 (s, 2H), 8.06 (s, 1H), 8.56 (d, 1H, J = 6.8 Hz), 10.90 (s, 1H). HPLC: (isocratic 20 % A, 80 % B), t_R = 15.06 min. MS (CI) m/z: 801 (MH^+), 799 (M^+ -H), 757 (MH^+ -CO_2), 709 (MH^+ -CH_2Ph). Anal. Calcd for $C_{41}H_{42}F_6N_4O_6$: C, 61.50; H, 5.29; N, 7.00. Found: C, 62.2; H, 5.65; N, 7.06.

*(5R)-Diastereoisomer (**2b**)*. Yield, 40 % (494 mg). mp 63–64°C. TLC: Rf (hexane–EtOAc, 5:5) = 0.5; IR (KBr) cm^{-1}: 1650 (C═O), 1685 (C═O), 1720 (C═O). ^{1}H NMR (DMSO-d_6) δ : 0.75–0.82 (m, 6H), 1.23–1.94 (m, 8H), 2.13–2.20 (m, 1H), 2.94–3.54 (m, 6H), 4.52–4.67 (m, 2H), 5.00 (d, 1H, J = 12.7 Hz), 5.06 (d, 1H, J = 12.6 Hz), 5.18 (d, 1H, J = 13.5 Hz), 5.25 (d, 1H, J = 13.5 Hz), 6.91 (t, 1H, J = 7.3 Hz), 7.03 (t, 1H, J = 7.3 Hz), 7.10 (s, 1H), 7.21 (bs, 1H), 7.25–7.31 (m, 5H), 7.68 (d, 1H, J = 6.9 Hz), 7.90 (s, 0.4H), 7.95 (s, 1.6H), 8.02 (s, 1H), 8.05 (d, 1H, J = 6.8 Hz), 10.84 (s, 1H); HPLC: (isocratic 20 % A, 80 % B), t_R = 21.50 min; MS (CI) m/z 801 (MH^+), 799 (M^+ -H), 757 (MH^+ -CO_2), 709 (MH^+ -CH_2Ph). Anal. Calcd for $C_{41}H_{42}F_6N_4O_6$: C, 61.50; H, 5.29; N, 7.00. Found: C, 61.60; H, 5.67; N, 6.81.

Pharmacology

Binding experiments were performed according to standard techniques (Cascieri et al 1992) using clones of Chinese Hamster Ovary (CHO) as the receptor source for both NK_1 and NK_2 subtypes. Crude membranes were prepared and stored in Tris 20 mM, sucrose 250 mM medium, pH 7.4, at –70°C. Both tritiated radio-antagonists were used with specific activities of 170 Ci $mmol^{-1}$ (Amersham). Incubation conditions were as follows: Tris 50 mM, Mg 2 mM (final concentrations), pH 7.4 and additional bacitracin 160 μg mL^{-1} at 25°C for 1 h. The reaction was terminated by rapid vacuum filtration onto glass fibre filters (GF/C Whatman pre-soaked for 2 h in PEI (polyethylnimine) 0.1 %): after 4 × 2-mL washes with Tris 50 mM at 4°C, pH 7.4, the radioactivity trapped

onto the filters was counted and the binding was calculated. Non-specific binding was determined with additional non-radioactive substance P 1 μM. Competition curves were fitted according to the Cheng and Prussoff equation (Kaleidagraph software, Microsoft for Macintosh) (Cheng & Prussoff 1973).

Results and Discussion

The synthetic route to [4,4]spirolactam derivatives (**1a**, **1b** and **2a**, **2b**) is outlined in Figure 2 and is an extension of the chemistry developed by Genin (1993) concerning [5,4]spirolactams. We have chosen a non-chiral route since the diastereoisomeric compounds (**1a**, **1b** and **2a**, **2b**) can be separated at the end of the synthesis by silica-gel column chromatography.

Fully protected DL-proline was alkylated with allyl bromide to give the α-allyl derivative **5**. Compound **5** was oxidized into aldehyde **6** which was then converted into the secondary amine **7** via a reductive amination with H-Leu-OMe·HCl. Deprotection of the *tert*-butyl ester function of **7** with trifluoroacetic acid resulted in the corresponding carboxylic acid as intermediate and cyclization into spirolactam was promoted by reflux in chloroform. The methoxycarbonyl function was next saponified by a methanolic hydroxide sodium solution to give carboxylic acid **9**. Finally, compounds **1** and **2** were obtained, respectively, by coupling between the carboxylic acid function of **9** and H-Trp-NH_2 or H-Trp-$OBzl(CF_3)_2$ (Millet et al 1999a) using PyBOP as a condensation agent. The diastereoisomers **1a** and **1b** (resp. **2a** and **2b**) were separated by silica-gel column chromatography and determination of the absolute stereochemistry of the spiro linkage of **1a** and **1b** (resp. **2a** and **2b**) was made by ^{1}H NMR. A convenient indicator of stable secondary structure, frequently applied in conformational analysis of proteins and peptides in water or organic solvents, is the temperature dependence of the amide NH ^{1}H chemical shift (Hinds et al 1991). An amide proton involved in a stable intramolecular hydrogen bond, or inaccessible to solvent for steric reasons, typically shows a reduced temperature coefficient of $> -6.0 \times 10^{-3}$ ppm K^{-1}. As was observed by Ward for GR71251 (Ward et al 1990), an intramolecular hydrogen bond (between NH of Trp and CO of Cbz) was apparent for **1a** and **2a,** the (*S*)-derivatives, due to the low value of the temperature coefficient of Trp-NH resonance ($\Delta\delta/\Delta T = -1.0 \times 10^{-3}$ ppm K^{-1} in DMSO-d_6 at 298–338 K for **1a** and **2a**). For **1b** and **2b**, ((*R*)- configuration), ^{1}H NMR studies failed to identify any intramolecular hydrogen bonding and suggested that they possessed an extended structure. Compounds **3** and **4** were obtained by standard methods of peptidic synthesis in solution (Boc chemistry) and precisely, for **4**, by the approach of Freidinger (Freidinger et al 1980, 1982) for the synthesis of γ-lactams. Values of the coefficients of Trp-NH resonance $\Delta\delta/\Delta T$ in DMSO-d_6 at 298–338 K were respectively: -2.8×10^{-3} ppm K^{-1} and -1.5×10^{-3} ppm K^{-1}. This suggested that **3** and **4** also presented a rigidified structure stabilized by H-bonds.

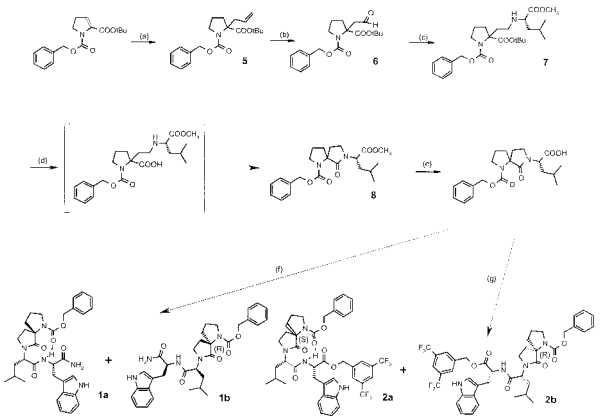

Figure 2 Synthesis of substituted spirolactams (**1**, **2**). Reagents and conditions: a, LDA, THF, allyl bromide, 4 h, −78°C; b, OsO_4, $NaIO_4$, THF, H_2O, 4 h, room temperature; c, H-Leu-OCH_3·HCl, NEt_3, Na $CNBH_3$, MeOH, 3-Å molecular sieves, 24 h, room temperature; d, 1. TFA, CH_2Cl_2, 24 h, room temperature, 2. NEt_3, $CHCl_3$, 24 h, reflux; e, NaOH, MeOH, 20 min, room temperature; f, H-Trp-NH_2, PyBOP, DIPEA. CH_2Cl_2, 48 h, room temperature; g, H-Trp-$OBzl(CF_3)_2$·HCl, PyBOP, DIPEA, 48 h, room temperature.

Table 1 NK_1 and NK_2 receptor binding

Compound	hNK_1 K_i^a (nM)	hNK_2 K_i^a (nM)
1a	> 10000	> 10000
1b	> 10000	> 10000
2a	1.6	> 10000
2b	126	> 10000
3	1.3	> 10000
4	4.0	> 10000
Substance P	0.16	139
Cbz-Gly-Leu-Trp-$OBzl(CF_3)_2$	40	250

[a]Inhibition of [^{3}H]substance P or [^{3}H]NKA specific binding to NK_1 or NK_2 receptors expressed in CHO cell lines (Cascieri et al 1992). Data represent the mean of triplicate determination (s.e. ± 10%).

The NK_1 and NK_2 receptor binding data is summarized in Table 1. Analysis of NK_1 and NK_2 affinities (Table 1) revealed that the introduction of $OBzl(CF_3)_2$ moiety into the spirolactam tryptophan ester compound

2a may have an important role in the interaction with NK_1 receptor when affinities of **2a** and **1a** are compared. Another element in NK_1 recognition was made apparent by introducing building units such as spirolactam, lactam or proline. These led to an increase in NK_1 affinity but not in NK_2 affinity, unlike that which occurred with Cbz-Gly-Leu-Trp-OBzl$(CF_3)_2$. The selectivity of **2a**, **3** and **4** for NK_1 receptors, when compared with that of Cbz-Gly-Leu-Trp-OBzl$(CF_3)_2$, seems to be in relation with the conformational restraints imposed by the geometrical features of their *N*-terminal sequence. Compounds **2a**, **3** and **4** would gain in affinity for the NK_1 receptor by virtue of greater entropy binding.

Conclusion

We have described the synthesis and structure–activity relationships for a series of analogues of spirolactam derivatives. In this case, our strategy consisted of synthesizing new compounds based on the C-terminal sequence of GR71251. This approach showed that the Trp-OBzl$(CF_3)_2$ moiety was essential for NK_1 affinity and that introducing building units leading to constrained peptides increased selectivity for NK_1receptors.

Conformational analyses of **2a**, **3** and **4** are ongoing. These compounds constitute a useful starting point for new substance P antagonists and represent attractive leads for further studies on metabolically more stable analogues.

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