Journal of Peptide Science
J. Peptide Sci. **7**: 323–330 (2001)
DOI: 10.1002/psc.326

Conformation of the Tripeptide Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ Deduced from Two-dimensional ^{1}H-NMR and Conformational Energy Calculations is Related to its Affinity for NK_1-Receptor

RÉGIS MILLET[1], LAURENCE GOOSSENS, JEAN-FRANÇOIS GOOSSENS[2], PHILIPPE CHAVATTE, KARINE BERTRAND-CAUMONT[3], RAYMOND HOUSSIN and JEAN-PIERRE HÉNICHART*

Institut de Chimie Pharmaceutique Albert Lespagnol, Université de Lille 2, 3 rue du Professeur Laguesse, B.P. 83, F-59006 Lille, France

Received 4 October 2000
Accepted 2 February 2001

Abstract: Chemical modifications of dual NK_1/NK_2 ligand Cbz-Gly-Leu-Trp-OBzl$(CF_3)_2$ (**1**) enabled us to create a high NK_1 selective ligand Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ (**2**). A determination of the conformational behavior of tripeptide **2** in solution is described. The 1D and 2D ^{1}H-NMR techniques (COSY and ROESY) were used to assign resonances. Observed interproton distance restraints were considered to characterize conformational behavior. Spectral data indicate that tripeptide **2** presents a rigidified structure in DMSO stabilized by H-bond in two γ-turns. Agreement with experimental data was obtained by averaging the ^{1}H-NMR parameters over several combinations of low-energy conformations. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: conformational analysis; neurokinin A; NMR; substance P

INTRODUCTION

Sensory neuropeptides (tachykinins) including substance P (SP: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) and neurokinin A (NKA: His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2) are co-released from capsaicin-sensitive primary afferent nerves by a variety of chemical, physical and pharmacological stimuli [1].

The central and peripheral actions of the mammalian tachykinin substance P (SP) have been associated with numerous diseases including migraine [2–5], rheumatoid arthritis [6–8], asthma [9,10] inflammatory bowel disease as well as with mediation of the emetic reflex [11,12], and with the modulation of central nervous system disorders such as Parkinson's disease [13] and depression [14,15].

Several NK_1 receptor antagonists, coming essentially from screening and optimization programs, have been described in recent years and some of them are being clinically developed [16–18].

We recently described [19] a potent NK_1/NK_2 ligand Cbz-Gly-Leu-Trp-OBzl$(CF_3)_2$ (**1**) whose peptidic structure was inspired from the *C*-terminal SP sequence. In order to optimize the affinity and the selectivity for NK_1 receptor, we replaced Gly by Pro since Pro is often observed at $(i+1)$ position of β-turn [20,21] and imposes strong restraints on the peptide conformation.

* Correspondence to: Institut de Chimie Pharmaceutique Albert Lespagnol, Université de Lille 2, 3 rue du Professeur Laguesse, B.P. 83, F-59006 Lille, France; e-mail: henicha@phare.univ-lille2.fr
[1] Current address: UMR 8525 CNRS-Université de Lille 2-Institut de Biologie et Institut Pasteur de Lille, 1 rue du Professeur Calmette, B.P. 447, F-59021 Lille, France.
[2] Current address: Laboratoire de Chimie Analytique, Faculté de Pharmacie, Université de Lille 2, 3 rue du Professeur Laguesse, B.P. 83, F-59006 Lille, France.
[3] Current address: GENFIT Parc Eurasanté, 256 rue Eugène Avinée, F-59120 Loos, France.

Evaluation of NK_1/NK_2 receptor binding affinity (Table 1) revealed tripeptide Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ (**2**) as a new selective NK_1 ligand with high affinity. For a better understanding of the interaction of **2** with its NK_1 receptor, we decided to determine its conformational behavior in DMSO-d_6 using the rotating frame Overhauser effect (ROESY) and molecular modeling.

MATERIALS AND METHODS

Peptide Synthesis

Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ was obtained (Figure 1) according to a previously described synthetic peptidic method in solution [22]. The cesium salt of *N*-Boc-Trp-OH was alkylated in DMF with 3,5-*bis*(trifluoromethyl)benzyl bromide. Removal of the Boc group of compound **3** in a methanolic HCl solution gave amine **4** which was reacted with Boc-Leu-OH using PyBOP as coupling agent to afford **5**. Deprotection of the Boc group using standard methodology resulted in amine **6**. Condensation with Cbz-Pro-OH gave the tripeptide Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ (**2**).

Table 1 NK_1 and NK_2 Receptor Binding Affinity

Compound	hNK_1 K_i^a (nM)	hNK_2 K_i^a (nM)
1	40	250
2	1.3	>10 000
SP	0.16	139

[a] Inhibition of [^{3}H]SP or [^{3}H]NKA specific binding to NK_1 or NK_2 receptors expressed in CHO cell lines. Data represents the mean of triplicate determination (S.E. ± 10%).

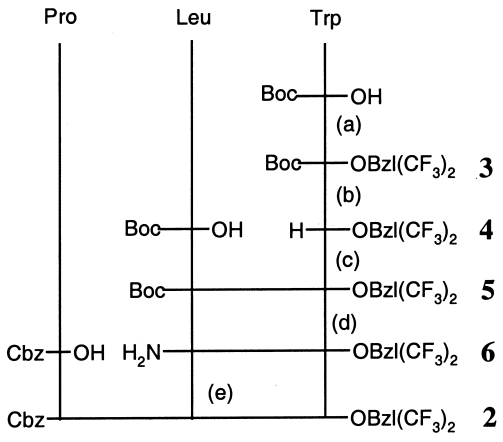


Figure 1 Reagents and conditions: (a) Cs_2CO_3, BrBzl$(CF_3)_2$, DMF; (b) MeOH, HCl; (c) PyBOP, DIEA, CH_2Cl_2; (d) MeOH, HCl; (e) PyBOP, DIEA, CH_2Cl_2.

Chemistry

Column chromatography was performed on silica gel 60 230–400 mesh purchased from Merck. Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ was characterized by MS, HPLC, elemental analysis, IR and NMR. Melting points were determined on a Büchi 535 capillary melting point apparatus and were uncorrected. Mass spectra were recorded on a Finnigan Mat SSQ 710 instrument in the electronic impact 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 determine the purity of the products. Elution was performed with the following two systems: 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). Elemental analyses for C, H, N were performed by the 'Service Central d'Analyses' at the CNRS, Vernaison, France, and were within 0.4% of theoretical values for the proposed structures. The IR spectra were determined as potassium bromide discs with a Perkin–Elmer 1310 spectrophotometer, absorbances were reported in ν (cm^{-1}). The spectra confirmed the proposed structures.

N-α-Cbz-Pro-Leu-Trp 3,5-Bis(trifluoromethyl)benzyl ester (2). Commercial Cbz-Pro-OH (249 mg, 1.0 mmol), *N*,*N*-diisopropylethylamine (0.51 ml, 3.0 mmol) and PyBOP (572 mg, 1.1 mmol) were added to a solution of H-Leu-Trp-OBzl$(CF_3)_2$. HCl [22] (543 mg, 1.0 mmol) in dichloromethane (40 ml). The reaction was stirred for 48 h. The solution was partitioned between dichloromethane and 1 N hydrochloric acid. The organic extract was dried (magnesium sulfate) and evaporated to yield an oil which was purified by column chromatography on silica gel using hexane/ethyl acetate (5/5). The precipitate thus obtained was recrystallized from dichloromethane/hexane (1/9) to give **2** as a white solid (55%); m.p. 65–67°C; IR (KBr) ν: 3380–3280 cm^{-1} (NH), 1750 cm^{-1} (CO), 1690 cm^{-1} (CO), 1645 cm^{-1} (CO); MS (electronic impact) m/z 774 (M), 666, 413, 130, 91; HPLC: (isocratic 10% A, 90% B), $\lambda = 220$, 230, 254 and 280 nM, $R_t = 11.25$ min, 100% purity. Anal. Calc. for $C_{39}H_{40}F_6N_4O_6$: C, 60.46; H, 5.20; N, 7.23; Found: C, 60.31; H, 5.25; N, 7.09%.

Pharmacology

Binding experiments were performed according to standard techniques [23] 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 20 mM Tris, 250 mM sucrose medium, pH 7.4, at -70°C. Both tritiated radioligands were used with specific activities of $\#170$ Ci/mmol (Amersham). Incubation conditions were the following: 50 mM Tris, 2 mM Mg (final concentrations), pH 7.4 and additional 160 μg/ml Bacitracine at 25°C for 60 min. The reaction was terminated by rapid vacuum filtration onto glass fiber filters (GF/C Whatman presoaked 2 h in 0.1% PEI): after four 2-ml washes with 50 mM Tris 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 Prusoff equation [24] (Kaleidagraph software, Microsoft for Macintosh).

Structure Calculations

Molecular modeling studies were performed using SYBYL [25] software version 6.4 running on a Silicon Graphics Indigo 2 R4600 workstation. Amides were considered in *trans* geometry. Three-dimensional models of Cbz-Pro-Leu-Trp-OBzl(CF_3)$_2$ were built from a library of standard fragments and their geometry was subsequently optimized using the Tripos force field [26] including the electrostatic term calculated from Gasteiger and Hückel atomic charges. The method of Powell available in the Maximin2 procedure was used for energy minimization until the gradient value was smaller than 0.001 kcal/mol/Å^2.

NMR Experiments

Peptides were dissolved in DMSO-d_6. ^{1}H-chemical shifts were given in parts per million (δ ppm) using tetramethylsilane as an internal standard. The ^{1}H-NMR spectra were recorded on a Bruker DPX 300 spectrometer equipped with a probe head broad band inverse, operating in the Fourier transform mode with quadrature detection at 300 MHz for protons. For ^{1}H-NMR spectra, typical acquisition parameters were 4000 Hz spectral width, 3.05 μs pulse width ($\pi/2 = 6.1$ μs) and 16 K time domain addresses. Assignments of the amino acid residues resonance were established by two-dimensional (2D) correlated spectroscopy using the pulse sequence D_1–90°–D_2–90° acquisition (COSY). ROESY experiments enabled us to show preferential conformational distance by constraints between pairs of protons. The pulse sequence was D_1–90°–D_2–(180°–180°)$_n$. Acquisition in the phase sensitive mode was used with a mixing time of 1 s through 320 experiments.

RESULTS AND DISCUSSION

Assignment Resonance of Amino Acid Residues of Cbz-Pro-Leu-Trp-OBzl(CF_3)$_2$

All ^{1}H-NMR assignments for Cbz-Pro-Leu-Trp-OBzl(CF_3)$_2$ in DMSO-d_6 at 293 K are reported in Table 2 including chemicals shifts and ^3J(NH–C^{z}H).

The COSY spectrum disclosed cross-peaks between δ and γ protons allowing for the unambiguous attribution of the protons of Leu and then Pro. Additional through-space sequential (NH$_i$–C^{z}H$_{i-1}$) connectivities resulted in complete resonance assignments. The ROESY spectrum (Figure 2) used to obtain distance constraints between pairs of protons gave the assignments of the aromatic protons of Trp via the cross-peaks between H_4 and β protons (Figure 3). The assignments of the other protons (3,5-*bis*(trifluoromethyl)benzyl and *N*-benzyloxycarbonyl) did not present any particular problem.

Conformational Behavior of Cbz-Pro-Leu-Trp-OBzl(CF_3)$_2$

The combined ROESY and molecular mechanics study was performed to characterize the bioactive putative conformation of Cbz-Pro-Leu-Trp-OBzl(CF_3)$_2$. The results led to three significant observations that enabled us to propose a conformational behavior of Cbz-Pro-Leu-Trp-OBzl(CF_3)$_2$.

Firstly, the presence of interresidue cross-peaks between aromatic ring protons of Cbz and C$^\delta$H (Leu) was detected (Figure 3). Conformational analysis of Cbz-Pro-Leu-Trp-OBzl(CF_3)$_2$ using molecular modeling studies revealed precisely that it was not possible to have a β-turn structure if such a connectivity cross-peaks is observed (Figure 4). Moreover, no cross-peaks connectivity between NH (Trp) and NH (Leu) were observed, while it is generally present in a β-turn arrangement [21]. These observations suggested that the peptide adopted an extended structure instead of a β-turn conformation. To verify these assumptions, the data derived

Table 2 ^{1}H Chemical Shifts of Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ in DMSO-d_6 at 293 K (10 mg/ml)

Residue	δ (ppm)							^{3}J(NH-C$^{\alpha}$H)
	NH	$\Delta\delta/\Delta T$ (ppb/K)	α	β	γ	δ	Other	(Hz)
Pro			4.20–4.24	1.61–1.81, 2.00–2.10	1.61–1.84	3.24–3.48		
Leu	8.04	−2.1	4.32	1.22–1.40	1.44–1.53	0.64–0.82		8.7
Trp	8.46	−2.8	4.53	3.11–3.20			(ring) NH 10.91; H_2 7.18; H_4 7.46; H_5 6.96; H_6 7.05; H_7 7.33	7.0
Cbz							4.90–5.06; (ring) 7.28–7.32	
Bzl$(CF_3)_2$							5.16–5.25; (ring) $H_{2'}$ and $H_{6'}$ 7.94; $H_{4'}$ 8.06	

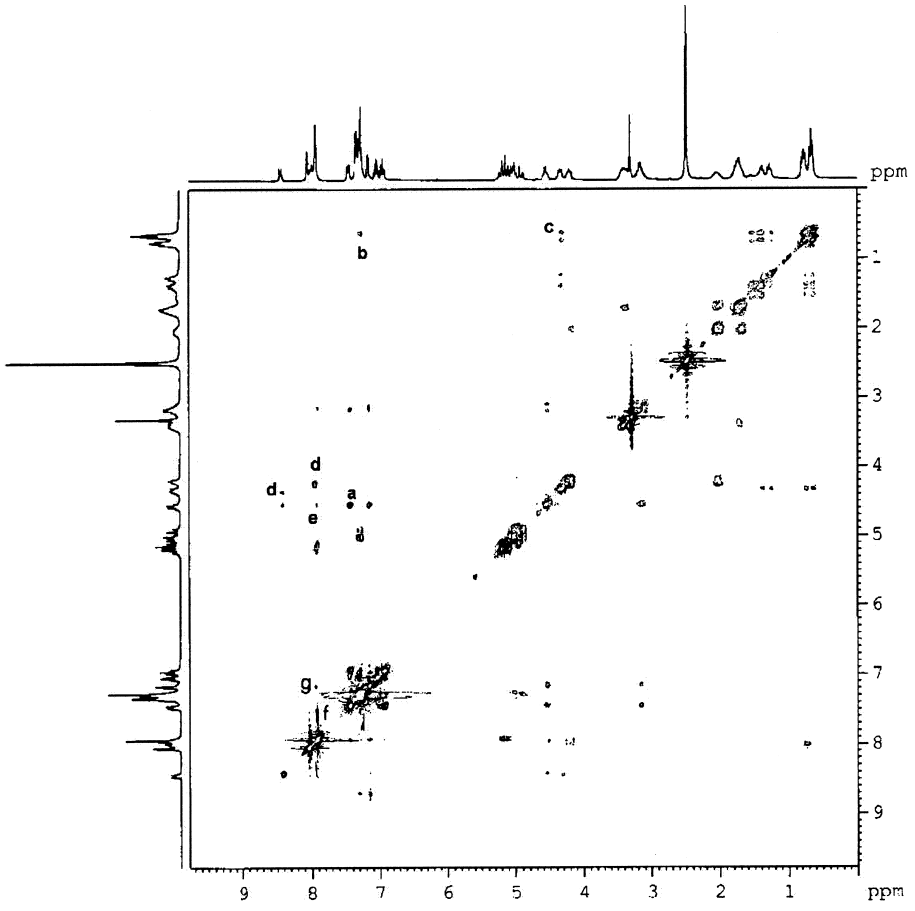
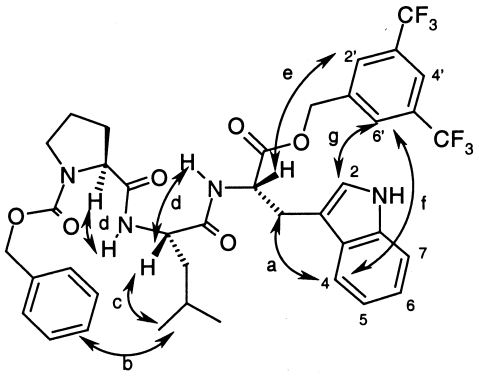


Figure 2 ROESY connectivity diagram recorded at 300 MHz of a 10 mg/ml Cbz-Pro-Leu-Trp-Obzl$(CF_3)_2$ solution in DMSO-d_6 showing selected interesidue cross-peaks a, b, c, d, e, f, g (for signification, see Figure 3).



	Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$
a	H_4 - $C^{\beta}H$ (Trp)
b	Cbz (ring) - $C^{\delta}H$ (Leu)
c	$C^{\alpha}H$ (Leu) - $C^{\delta}H$ (Leu)
d	$C^{\alpha}H$ (Pro) - NH (Leu)
	$C^{\alpha}H$ (Leu) - NH (Trp)
e	$C^{\alpha}H$ (Trp) - $H_{2'}$ and / or $H_{6'}$
f	H_4 - $H_{2'}$ and / or $H_{6'}$
g	H_2 - $H_{2'}$ and / or $H_{6'}$

Figure 3 Representation of selected ROESY connectivities observed for Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$. The arrows indicate connectivities observed in the ROESY spectrum.

from ROESY was used to build models or to serve as inputs in constraint molecular dynamic studies. The NMR constraints previously obtained were employed for the selection of the tripeptide starting conformations. For this purpose, we used the information on the connectivity between the protons of the Cbz ring with that of the $C^{\delta}H$ (Leu). The interaction between $C^{\delta}H$ (Leu) and $C^{\varepsilon}H$ (Leu) was also considered. These elements reduced the number of *N*-terminal sequence conformations. Thus, an ordered γ-turn structure consisting of a folded segment stabilized by a putative hydrogen bond between NH (Leu) and the CO of Cbz was postulated. Energy minimization of Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ allowed the next NH (Trp) to form a second γ-turn via an hydrogen bond with Leu. Interestingly, the conformation obtained after energy minimization positioned Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ into a rigidified *N*-terminal structure with two intramolecular hydrogen bonds. There was no evidence of any other stable structural features (Figure 5) in the Cbz-Pro-Leu-Trp segment.

The dual γ-turn structure proposition was deduced using amide chemical shift dependence on temperature: $\Delta\delta/\Delta T$. Both Leu and Trp had a low value temperature coefficient (> -3.0 ppb/K) in DMSO-d_6 at 298–338 K, which strongly supported the dual γ-turn suggestion.

Secondly, ROESY cross-peaks between $C^{\varepsilon}H$ (Pro)-NH (Leu) and $C^{\varepsilon}H$ (Leu)-NH (Trp) (Figure 3) were observed. Both elements concorded with the proposition of two γ-turns in the *N*-terminal moiety and allowed a trans conformation of Leu and Trp.

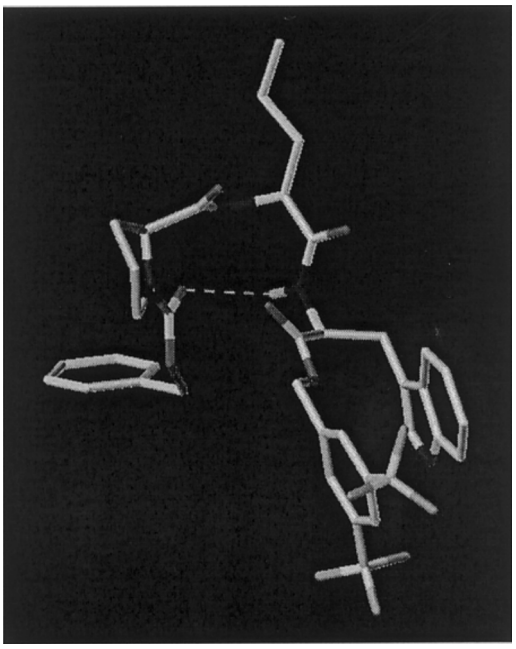


Figure 4 Stereoviews of a simulated β-turn Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ conformation.

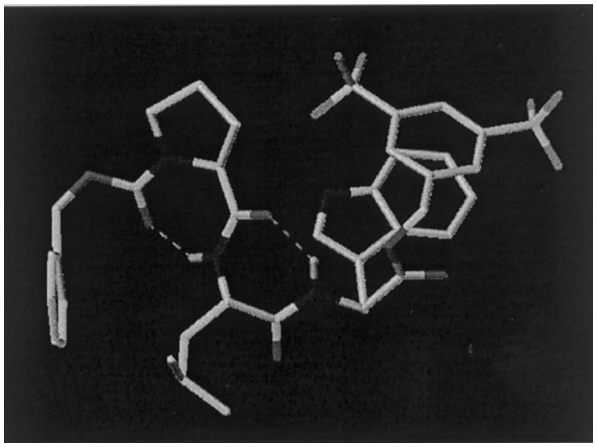


Figure 5 Stereoviews of Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ calculated from simulated annealing and according to the ROESY connectivity data. H-bond and putative aromatic interactions are noted.

Thirdly, the results showed that the *C*-terminal sequence exhibited an intramolecular $\pi-\pi$ interaction between the 3,5-*bis*(trifluoromethyl)benzyl group and indole moiety. Interresidue cross-peaks displayed connectivities between H_2 (indole), H_4 (indole), and $C^{\alpha}H$ (Trp) with $H_{2'}$ and/or $H_{6'}$. These observations indicated clearly that these two aromatic moieties, which are essential for NK_1 affinity [19], represent a global U-shaped pharmacophore. Moreover, as regards the aromatic interactions between the 3,5-*bis*(trifluoromethyl)benzyl group and indole moiety, the lowest energy calculation fitted with a face-to-face $\pi-\pi$ interaction instead of an edge-to-face interaction. The orientation of the *C*-terminal sequence was in accordance with all observed ROESY connectivities.

The energy conformation between putative bioactive conformation (Figure 5) and a β-turn simulated conformation (Figure 4) was next compared. It was observed that the supposed conformation had lower energy ($\Delta E = 3.78$ kcal/mol) than the β-turn conformation ($\Delta E = 13.5$ kcal/mol).

Finally, to validate this study, a representative sample of the conformational space was generated by a 100-cycle simulated annealing experiment. Computation begun at 900 K and the system was held at that temperature for 2000 fs. The temperature was then reduced with exponential ramping until 300 K was reached. At this point, the conformation was noted for energy minimization. Ten conformations of Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ with an acceptable energy conformation ($\Delta E < 10$ kcal/mol) were selected. Conformation diversity was considerable, but only one structure concorded with the ROESY connectivities observed, which proved to be the conformation previously calculated (Figure 5).

Structure–Activity Relationships

Evaluation of NK_1/NK_2 receptor binding affinity (Table 1) revealed that the replacement of a Gly residue by Pro in the Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ (**2**) increased affinity and selectivity for NK_1 receptor. Conformational analysis of the tripeptide Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ deduced from combined NMR spectroscopy and molecular mechanics calculations enabled us to discuss the structure–function relationship with the NK_1 receptor.

In a previous report [19] concerning the 3,5-*bis*(trifluoromethyl)benzyl ester of tryptophan (L-732 138), a competitive and selective NK_1 antagonist ($IC_{50} = 1.6$ nM for NK_1), we reported a putative three-point pharmacophoric interaction between the NK_1 receptor and the antagonist. This interaction was imposed by indole residue, benzyl group and ester linkage via the carbonyl group (Figure 6). These elements established a global U-shaped pharmacophore favored by a face-to-face $\pi-\pi$ interaction between the 3,5-*bis*(trifluoromethyl)benzyl group and indole moiety, which were both implicated in the interaction with two residues His^{265} and His^{197} of the NK_1 receptor [27,28].

All the results obtained concerning the *C*-terminal sequence of Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ were in total agreement with those observed and led to the postulation that the *C*-terminal sequence was responsible for NK_1 affinity. The direct influence of the *N*-terminal sequence on the increased affinity for the NK_1 receptor seemed difficult to correlate with the structure of tripeptide **1**. The identification of Cbz-Gly-Leu-Trp-OBzl$(CF_3)_2$ (**1**) as a lead compound [22] in the conception of dual NK_1/NK_2 ligand led us to explore the effect of chemical modifications in the amino acid side-chain and proved that the indolylmethyl and the Cbz carbamate groups were essential for NK_2 affinity. Replacement of a Gly residue by Pro in **1** made it possible to obtain a high selective NK_1 ligand. The suppression of NK_2 affinity seems to be related to the fact that *N*-terminal sequence was arranged in a rigid structure and that the Cbz ring interacted with $C^{\delta}H$ (Leu), thus impeding any interaction.

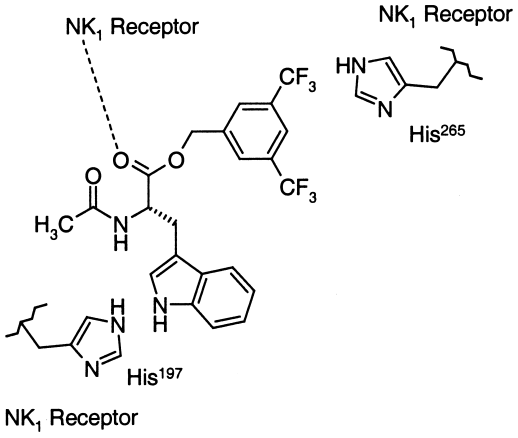


Figure 6 Representation of a proposed pharmacophoric receptor interaction of tryptophan ester NK_1 antagonist L-732 138 based on SAR data obtained from site-directed mutagenesis of the NK_1 receptor.

CONCLUSION

The synthesis of a suitable NK_1 ligand has been described and characterized. The three-dimensional structure of Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ was studied by one and two-dimensional 300 MHz NMR experiments in DMSO and by molecular modeling. The results indicate that peptide **2** did not adopt a β-turn arrangement as expected on the basis of a previous report [21], but an extended conformation stabilized by a H-bond in two γ-turns. Together, the results show that the *C*-terminal architecture was stabilized by a face-to-face interaction between the 3,5-*bis*(trifluoromethyl)benzyl group and indole moiety. These two elements, which are essential for NK_1 affinity, concorded with our previous work and with results reported by other researchers [27–29]. The determination of the three-dimensional bioactive conformation of Cbz-Pro-Leu-Trp-OBzl$(CF_3)_2$ could be applied in the conception of non-peptidic NK_1 antagonists of direct therapeutic interest.

REFERENCES

1. Maggi CA, Pataccini R, Rovero P, Giachetti A. Tachykinin receptors and tachykinin receptor antagonists. *J. Auton. Pharmacol.* 1993; **13**: 23–93.
2. Beattie DT, Connor HE, Hagan RM. Recent developments in tachykinin NK_1 receptor antagonists: prospects for the treatment of migraine headache. *Can. J. Physiol. Pharmacol.* 1995; **73**: 871–877.
3. Carmody J, Pawlak M, Messlinger K. Lack of a role for substance P in the control of dural arterial flow. *Exp. Brain Res.* 1996; **111**: 424–428.
4. Moskowitz MA, Buzzi MGJ. Neuroeffector functions of sensory fibres: implications for headache mechanisms and drug actions. *Neurology* 1991; **238**: S18–S22.
5. Polley JS, Gaskin PJ, Perren MJ, Connor HE, Ward P, Beattie DT. The activity of GR205171, a potent non-peptide tachykinin NK_1 receptor antagonist, in the trigeminovascular system. *Regul. Peptides* 1997; **68**: 23–29.
6. Bozic CR, Lu B, Hopken UE, Gerard C, Gerard NP. Neurogenic amplification of immune complex inflammation. *Science* 1996; **273**: 1722–1725.
7. Levine JD, Clark R, Devor M, Helms C, Moskowitz MA, Basbaum AI. Intraneuronal substance P contributes to the severity of experimental arthritis. *Science* 1984; **226**: 547–549.
8. Krause JE, Di Maggio DA, McCarson KE. Alterations in neurokinin 1 receptor gene expression in models of pain and inflammation. *Can. J. Physiol. Pharmacol.* 1995; **73**: 854–859.
9. Naline E, Molimard M, Regoli D, Emonds-Alt X, Bellamy JF, Advenier C. Evidence for functional tachykinin NK_1 receptors on human isolated small bronchi. *Am. J. Physiol.* 1996; **271**: L763–767.
10. Ollerenshaw SL, Jarvis D, Sullivan CE, Woolcock AJ. Substance P immunoreactive nerves in airways from asthmatics and nonasthmatics. *Eur. Resp. J.* 1991; **4**: 673–682.
11. Singh L, Field MJ, Hughes J, Kuo BS, Suman-Chauhan N, Tuladhar BR, Wright DS, Naylor RJ. The tachykinin NK_1 receptor antagonist PD 154075 blocks cisplatin-induced delayed emesis in the ferret. *Eur. J. Pharmacol.* 1997; **321**: 209–216.
12. Ward P, Armour DR, Bays DE, Evans B, Giblin GMP, Heron N, Hubbard T, Liang K, Middlemiss D, Mordaunt J, Naylor A, Pegg NA, Vinader MV, Watson SP, Bountra C, Evans DC. Discovery of an orally bioavailable NK_1 receptor antagonist, (2*S*,3S)-(2-methoxy-5-tetrazol-1-ylbenzyl)(2-phenylpiperidin-3-yl)amine (GR203040), with potent antiemetic activity. *J. Med. Chem.* 1995; **38**: 4985–4992.
13. Whitty CJ, Walker PD, Goebel DJ, Poosch MS, Bannon MJ. Quantitation, cellular localization and regulation of neurokinin receptor gene expression within the rat substantia nigra. *Neuroscience* 1995; **64**: 419–425.
14. Wahlestedt C. Reward for persistence in substance P research. *Science* 1998; **281**: 1624–1625.
15. Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, Reines SA, Liu G, Snavely D, Wyatt-Knowles E, Hale JJ, Mills SG, MacLoss M, Swain CJ, Harrison T, Hill RG, Hefti F, Scolnick EM, Cascieri MA, Chicchi GG, Sadowski S, Williams AR, Hewson L, Smith D, Carlson EJ, Hargreaves RJ, Rupniak NM. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* 1998; **281**: 1640–1645.
16. McLean S. Nonpeptide antagonists of the NK_1 tachykinin receptor. *Med. Res Rev.* 1996; **16**: 297–317.
17. Quartara L, Maggi CA. The tachykinin NK_1 receptor. Part I: ligands and mechanisms of cellular activation. *Neuropeptides* 1997; **3**: 537–563.
18. Quartara L, Maggi CA. The tachykinin NK_1 receptor. Part II: Distribution and pathophysiological roles. *Neuropeptides* 1998; **32**: 1–49.
19. Millet R, Goossens J-F, Bertrand-Caumont K, Houssin R, Hénichart J-P. Synthesis and biological evaluation of conformationally restricted derivatives of tryptophan as NK_1/NK_2 ligands. *Lett. Peptide Sci.* 1999; **6**: 221–233.
20. Hanesssian S, McNaughton-Smith G, Lombart HG, Lubell WD. Design and synthesis of conformationally constrained amino acids as versatile scaffolds and peptide mimetics. *Tetrahedron* 1997; **38**: 12789–12854.
21. Hayashi T, Assi T, Ogoshi H. Conformational analysis of β-turn structure in tetrapeptides containing proline

or proline analogs. *Tetrahedron Lett*. 1997; **38**: 3039–3042.
22. Millet R, Goossens JF, Bertrand-Caumont K, Houssin R, Hénichart JP. Synthesis and biological evaluation of tripeptide derivatives of Cbz-Gly-Leu-Trp-OBzl$(CF_3)_2$. *Lett. Peptide Sci*. 1999; **6**: 255–262.
23. Cascieri MA, Ber E, Fong TM, Sadowski S, Bansal A, Swain C, Seward E, Frances B, Burns D, Strader CD. Characterization of the binding of a potent, selective, radioiodinated antagonist to the human NK_1 receptor. *Mol. Pharmacol*. 1992; **42**: 458–463.
24. Cheng Y, Prusoff WH. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol*. 1973; **22**: 3099–3108.
25. SYBYL 6.4, Tripos Associates, Inc., St. Louis, MO.
26. Marot C, Chavatte P, Morin-Allory L, Viaud MC, Guillaumet G, Renard P, Lesieur D, Michel A. Pharmacophoric search and 3D-QSAR comparative molecular field analysis studies on agonists of melatonin sheep receptors. *J. Med. Chem*. 1998; **41**: 4453–4465 and references therein.
27. Lewis RT, MacLeod AM, Merchant KJ, Kellerher F, Sanderson I, Herbert RH, Cascieri MA, Sadowski S, Ball RG, Hoogsteen K. Tryptophan-derived NK_1 antagonists: conformationally constrained heterocyclic bioisosteres of the ester linkage. *J. Med. Chem*. 1995; **38**: 923–933.
28. Cascieri MA, MacLeod AM, Underwood D, Shiao LL, Ber E, Sadowski S, Yu H, Merchant KJ, Swain C, Strader CD, Fong TM. Characterization of the interaction of *N*-acyl-L-tryptophan benzyl ester neurokinin antagonists with the human NK_1 receptor. *J. Biol. Chem*. 1994; **269**: 6587–6591.
29. MacLeod AM, Merchant KJ, Brookfield F, Kelleher F, Stevenson G, Owens AP, Swain CJ, Cascieri MA, Sadowski S, Ber E, Strader CD, MacIntyre DE, Metzger JM, Ball RG, Baker R. Identification of L-tryptophan derivatives with potent and selective antagonist activity at the NK_1 receptor. *J. Med. Chem*. 1994; **37**: 1269–1274.

